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**UTILITY  
PATENT APPLICATION  
TRANSMITTAL**

(Only for new nonprovisional applications under 37 CFR 1.53(b))

Attorney Docket No.	BB1334 USNA CNT1
First Named Inventor or Application Identifier	RICHARD MARTIN BROGLIE
Express Mail Label No.	EL073739282US
Express Mailing Date	AUGUST 22, 2000

**APPLICATION ELEMENTS**

See MPEP chapter 600 concerning utility patent application contents.

**ADDRESS TO:** Assistant Commissioner for Patents  
Box Patent Application  
Washington, DC 20231

- |  |  |
|--|--|
| <p>1. <input checked="" type="checkbox"/> Fee (Authority to charge deposit account below.)<br/>(Submit an original, and a duplicate for fee processing)</p> <p>2. <input checked="" type="checkbox"/> Specification [Total Pages <input ]<br="" type="text" value="60"/>(preferred arrangement set forth below)</p> <ul style="list-style-type: none"><li>- Descriptive title of the invention</li><li>- Cross References to Related Applications (if needed)</li><li>- Statement Regarding Fed sponsored R &amp; D (if needed)</li><li>- Reference to Microfiche Appendix (if filed)</li><li>- Background of the Invention</li><li>- Brief Summary of the Invention</li><li>- Brief Description of the Drawings (if filed)</li><li>- Detailed Description</li><li>- Claim(s)</li><li>- Abstract of the Disclosure</li></ul> <p>3. <input checked="" type="checkbox"/> Drawing(s) (35 USC 113) [Total Sheets <input ]<="" p="" type="text" value="4"/><p>4. <input checked="" type="checkbox"/> Oath or Declaration [Total Pages <input ]<="" p="" type="text" value="1"/><ul style="list-style-type: none"><li>a. <input type="checkbox"/> Newly executed (original or copy)</li><li>b. <input checked="" type="checkbox"/> Copy from a prior application (37 CFR 1.63(d))<br/>(for continuation/divisional with Box 14 completed)</li><li>i. <input type="checkbox"/> <b>DELETION OF INVENTORS</b><br/>Signed Statement below at 15 deleting inventor(s) named in the prior application, see 37 CFR 1.63(d)(2) and 1.33(b).</li></ul><p>5. <input checked="" type="checkbox"/> Incorporation by Reference (useable if Box 4b is checked)<br/>The entire disclosure of the prior application, from which a copy of the oath or declaration is supplied under Box 4b, is considered as being part of the disclosure of the accompanying application and is hereby incorporated by reference therein.</p></p></p> | <p>6. <input type="checkbox"/> Microfiche Computer Program (Appendix)</p> <p>7. Nucleotide and/or Amino Acid Sequence Submission (if applicable, all necessary)</p> <ul style="list-style-type: none"><li>a. <input checked="" type="checkbox"/> Computer Readable Copy</li><li>b. <input checked="" type="checkbox"/> Paper Copy (identical to computer copy)</li><li>c. <input checked="" type="checkbox"/> Statement verifying identity of above copies</li></ul> |
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**ACCOMPANYING APPLICATION PARTS**

8. ☐ Power of Attorney
9. ☒ Information Disclosure Statement (IDS)/Cover Letter plus PTO-1449 ☐ Copies of IDS Citations
10. ☒ Preliminary Amendment
11. ☒ Return Receipt Postcard (MPEP 503)  
(Should be specifically itemized)
12. ☐ Certified Copy of Priority Document(s)  
(if foreign priority is claimed)
13. ☐ Other:

**14. If a CONTINUING APPLICATION, check appropriate box and supply the requisite information:**☒ Continuation ☐ Divisional ☐ Continuation-in-part (CIP) of prior Application No.: 09/232,948

15. ☐ **DELETION OF INVENTOR(S) STATEMENT:** This application is being filed by less than all the inventors named in the prior application. In accordance with 37 CFR 1.63(d)(2) and 1.33(b), the Assistant Commissioner is requested to delete the name(s) of the following person or persons who are not inventors of the invention being claimed in this application:
16. ☒ Amend the specification by inserting before the first line the sentence:  
-- This is a continuation of Application No. 09/232,948 filed January 19, 1999, now pending which is a continuation of Application No. 08/728,025 filed October 9, 1996, now abandoned. --
17. ☐ Cancel in this application original claims \_\_\_\_ of the prior application before calculating the filing. (At least one original independent claim must be retained for filing purposes.)
18. ☐ Priority of foreign Application No. \_\_\_\_\_ filed on \_\_\_\_\_ in \_\_\_\_\_  
\_\_\_\_\_ is claimed under 35 U.S.C. 119.  
(country)

CLAIMS	(1) FOR	(2) NUMBER FILED	(3) NUMBER EXTRA	(4) RATE	(5) CALCULATIONS
	<b>TOTAL CLAIMS</b> (37 CFR 1.16(c))	36 - 20 =	16	x \$ 18 =	\$ 288.00
	<b>INDEPENDENT CLAIMS</b> (37 CFR 1.16(b))	3 - 3 =	0	x \$ 78 =	0
	<b>MULTIPLE DEPENDENT CLAIM(S) (if applicable)</b>			+ \$ 260 =	0
				<b>BASIC FEE</b> (37 CFR 1.16(a))	+ \$ 690.00
				<b>TOTAL =</b>	<b>\$ 978.00</b>

19. The Commissioner is hereby authorized to credit overpayments or charge the following fees to Deposit Account No. 04-1928:

a. ☒ Fees required under 37 CFR 1.16.

b. ☒ Fees required under 37 CFR 1.17.

20. ☐ Other:

#### 21. CORRESPONDENCE ADDRESS

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#### 22. SIGNATURE OF ATTORNEY OR AGENT REQUIRED

NAME	Lynne M. Christenbury	REG. NO.: 30,971
SIGNATURE	<i>Lynne M. Christenbury</i>	
DATE	22 August 2000	

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

IN THE APPLICATION OF:

RICHARD MARTIN BROGLIE ET AL. CASE NO.: BB1334 USNA CNT1

APPLN. NO.: UNKNOWN GROUP ART UNIT: UNKNOWN

FILED: CONCURRENTLY HEREWITH EXAMINER: UNKNOWN

FOR: GENES FOR MUTANT MICROSOMAL DELTA-12  
FATTY ACID DESATURASES AND RELATED  
ENZYMES FROM PLANTS

Date: AUGUST 22, 2000

Assistant Commissioner for Patents  
Washington, DC 20231

Sir:

**Preliminary Amendment**

This is submitted to facilitate prosecution of the above-identified application.

**In the Claims**

Kindly amend the following claims:

1. (Amended) A plant containing a recombinant nucleic acid construct, said construct comprising at least one seed-specific regulatory sequence operably linked in sense orientation to a mutant delta-12 fatty acid desaturase gene encoding a [protein] delta-12 fatty acid desaturase gene product having [a] at least one mutation which renders said desaturase gene product non-functional, said mutation being in a [His-(Asp/Glu)-Cys-(Gly/Ala)-His] His - X - Cys - Y - His (SEQ ID NO:17) amino acid region wherein X is selected from the group consisting of Asp and Glu and Y is selected from the group consisting of Gly and Ala, and further wherein said construct confers [altered fatty acid composition] decreased linoleic acid content in seeds of said plant.

4. (Amended) The plant of claim 3, wherein seeds of said [altered fatty acid composition] plant comprise [comprises] from about 1.0% to about 10.0% linoleic acid, based on total fatty acid composition.

5. (Amended) The plant of claim 1, wherein seeds of said [altered fatty acid composition] plant comprise [comprises] from about 69% to about 90% oleic acid, based on total fatty acid composition.

6. (Amended) The plant of claim 1, wherein said mutant desaturase gene encodes a microsomal delta-12 fatty acid desaturase gene product.

29. (Amended) A recombinant nucleic acid construct effective for [altering fatty acid composition] decreasing linoleic acid content in seeds, said construct comprising at least one seed-specific regulatory sequence operably linked in sense orientation to a mutant delta-12 fatty acid desaturase encoding a delta-12 fatty acid desaturase gene product having at least one mutation which renders said desaturase gene product non-functional, said mutation being in a His - X - Cys - Y - His (SEQ ID NO:17) amino acid region wherein X is selected from the group consisting of Asp and Glu and Y is selected from the group consisting of Gly and Ala.

#### In the Specification

Kindly amend the specification as follows:

At page 7 at line 18, please add -- SEQ ID NO:17 is a 5 amino acid sequence in which at least one mutation in the conserved three amino acids of this motif renders the delta-12 desaturase gene product non-functional.--

At page 12, line 33, please delete "nuclidotides" and insert therefor -- nucleotides--.

At page 18, line 19, please delete "enzyme" and insert therefor --mutant--.

At page 28, line 21, please delete "(Table 5)", and substitute therefor -- (Table 2)--.

At page 29, in Table 2, middle column, please underline E and G in lines 1 and 2. Please underline D and A in line 3. Please underline D and G in lines 4 and 5.

At page 37, line 28, please delete "6 and 7", and substitute therefor --3 and 4--.

At page 38, line 29, please delete "6", and substitute therefor --3--.

#### Remarks

This case is a continuation under 37 CFR §1.53(b) of Application No. 09/232,948 filed on January 19, 1999.

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The claims have been clarified to recite that the microsomal gene product is the delta-12 enzyme. In addition, claim 1 now also recites that there is at least one mutation in the cited region which renders the delta-12 desaturase gene product non-functional. Support for the motif (including conservative substitutions) recited in claim 1 can be found in Table 2 on page 27 of the specification.

The claims have been amended to recite that the linoleic fatty acid content is decreased and that non-functional mutants are claimed.

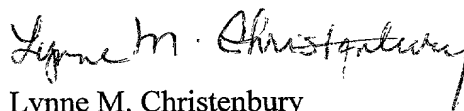
Submitted herewith are an updated Sequence Listing and a substitute paper copy. Support for SEQ ID NO:17 can be found in Table 2 on page 29. Thus, no new matter has been added.

The Brief Description of the Sequence Descriptions section of the specification has been amended to refer to SEQ ID NO: 17.

Enclosed herewith along with this Preliminary Amendment is an Information Disclosure Statement setting forth all references which had been cited by Applicants or the Examiner in connection with Serial No. 09/232,948 and some additional information as well as a petition for a three (3) month extension of time. Also submitted herewith are exact copies of papers that were submitted with the parent application (Serial No. 08/728,025) to correct inventorship. The original papers can be found in the parent file.

Please charge any fees which are required in connection with the filing of this Preliminary Amendment, Information Disclosure Statement, Declaration in Accordance With 37 CFR 1.821, and Petition for Extension of Time to Deposit Account No. 04-1928 (E. I. du Pont de Nemours and Company).

Respectfully submitted,



Lynne M. Christenbury  
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Enclosures

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TITLEGENES FOR MUTANT MICROSOMAL DELTA-12 FATTY ACID  
DESATURASES AND RELATED ENZYMES FROM PLANTSFIELD OF THE INVENTION

5       The invention relates to the preparation and use of  
nucleic acid fragments encoding fatty acid desaturase  
enzymes to modify plant lipid composition. Chimeric genes  
incorporating such nucleic acid fragments and suitable  
regulatory sequences may be used to create transgenic  
10   plants with altered levels of unsaturated fatty acids.

BACKGROUND OF THE INVENTION

Plant lipids have a variety of industrial and  
nutritional uses and are central to plant membrane function  
and climatic adaptation. These lipids represent a vast  
15   array of chemical structures, and these structures  
determine the physiological and industrial properties of  
the lipid. Many of these structures result either directly  
or indirectly from metabolic processes that alter the  
degree of unsaturation of the lipid. Different metabolic  
20   regimes in different plants produce these altered lipids,  
and either domestication of exotic plant species or  
modification of agronomically adapted species is usually  
required to economically produce large amounts of the  
desired lipid.

25       Plant lipids find their major use as edible oils in the  
form of triacylglycerols. The specific performance and  
health attributes of edible oils are determined largely by  
their fatty acid composition. Most vegetable oils derived  
from commercial plant varieties are composed primarily of  
30   palmitic (16:0), stearic (18:0), oleic (18:1), linoleic  
(18:2) and linolenic (18:3) acids. Palmitic and stearic  
acids are, respectively, 16- and 18-carbon-long, saturated  
fatty acids. Oleic, linoleic, and linolenic acids are 18-  
carbon-long, unsaturated fatty acids containing one, two,  
35   and three double bonds, respectively. Oleic acid is  
referred to as a mono-unsaturated fatty acid, while  
linoleic and linolenic acids are referred to as poly-  
unsaturated fatty acids.

Many recent research efforts have examined the role that saturated and unsaturated fatty acids play in reducing the risk of coronary heart disease. In the past, it was believed that mono-unsaturates, in contrast to saturates and poly-unsaturates, had no effect on serum cholesterol and coronary heart disease risk. Several recent human clinical studies suggest that diets high in mono-unsaturated fat and low in saturated fat may reduce the "bad" (low-density lipoprotein) cholesterol while maintaining the "good" (high-density lipoprotein) cholesterol (Mattson et al., *Journal of Lipid Research* (1985) 26:194-202).

A vegetable oil low in total saturates and high in mono-unsaturates would provide significant health benefits to consumers as well as economic benefits to oil processors. For specialized uses, high levels of poly-unsaturates can be desirable. Linoleate and linolenate are essential fatty acids in human diets, and an edible oil high in these fatty acids can be used for nutritional supplements, for example in baby foods.

The biosynthesis of the major plant lipids has been the focus of much research (Browse et al., *Ann. Rev. Plant Physiol. Mol. Biol.* (1991) 42:467-506). These studies show that, with the notable exception of the soluble stearoyl-acyl carrier protein desaturase, the controlling steps in the production of unsaturated fatty acids are largely catalyzed by membrane-associated fatty acid desaturases. Desaturation reactions occur in plastids and in the endoplasmic reticulum using a variety of substrates including galactolipids, sulfolipids, and phospholipids. Genetic and physiological analyses of Arabidopsis thaliana nuclear mutants defective in various fatty acid desaturation reactions indicates that most of these reactions are catalyzed by enzymes encoded at single genetic loci in the plant. These investigations have demonstrated the role of delta-12 desaturase and delta-15 desaturase activities in the production of linoleate and linolenate from 2-oleoyl-phosphatidylcholine and

2-linoleoyl-phosphatidylcholine, respectively (Wang et al.,  
*Plant Physiol. Biochem.* (1988) 26:777-792). Thus,  
modification of the activities of these enzymes represents  
an attractive target for altering the levels of lipid  
unsaturation by genetic engineering.

The cloning and characterization of wild-type delta-12  
fatty acid desaturases has been reported (Okuley, et al.,  
*Plant Cell* (1994) 6:147-158). However, there are no  
teachings concerning plants having seed-specific expression  
of mutant delta-12 or delta-15 fatty acid desaturase gene  
products. Furthermore, no methods have been described for  
altering the fatty acid composition of plants using nucleic  
acid constructs expressing a mutant delta-12 or a mutant  
delta-15 fatty acid desaturase.

#### SUMMARY OF THE INVENTION

Applicants have discovered a means to control the  
nature and levels of unsaturated fatty acids in plants.  
Nucleic acid fragments from cDNAs or genes encoding mutant  
fatty acid desaturases are used to create chimeric genes.  
The chimeric genes may be used to transform various plants  
to modify the fatty acid composition of the plant or the  
oil produced by the plant. The invention comprises nucleic  
acid constructs containing mutant microsomal delta-12 or  
mutant microsomal delta-15 fatty acid desaturase coding  
sequences, which are operably linked in sense orientation  
to at least one regulatory sequence. Such a construct is  
effective for altering fatty acid composition of seeds when  
the construct is introduced into a plant. In one  
embodiment, a mutant coding sequence for a delta-12 fatty  
acid desaturase comprises the mutation in the sequence of  
SEQ ID NO:3.

The invention further comprises seeds, plants and plant  
lines having a recombinant nucleic acid construct  
containing at least one regulatory sequence linked in sense  
orientation to a mutant delta-12 or mutant delta-15 fatty  
acid desaturase. The mutant chimeric gene preferentially  
is expressed in seeds and results in an altered fatty acid  
composition in seeds of such plants. A plant expressing a



mutant delta-12 desaturase gene preferably has a reduced level of linoleic acid in seeds. A plant expressing a mutant delta-15 desaturase gene preferably has a reduced level of  $\alpha$ -linolenic acid in seeds. If desired, a plant of  
5 the invention may express both a mutant delta-12 and a mutant delta-15 fatty acid desaturase, resulting in the reduction of both linoleic acid and  $\alpha$ -linolenic acid in seeds.

Yet another embodiment of the invention involves a  
10 method of producing seed oil containing altered levels of unsaturated fatty acids comprising: (a) transforming a plant cell with a chimeric gene described above;  
(b) growing sexually mature plants from the transformed plant cells of step (a); (c) screening progeny seeds from  
15 the sexually mature plants of step (b) for the desired levels of unsaturated fatty acids, and (d) processing the progeny seed of step (c) to obtain seed oil containing altered levels of the unsaturated fatty acids. Preferred plant cells and oils are derived from soybean, rapeseed,  
20 sunflower, cotton, cocoa, peanut, safflower, coconut, flax, oil palm, and corn. Preferred methods of transforming such plant cells would include the use of Ti and Ri plasmids of Agrobacterium, electroporation, and high-velocity ballistic bombardment.

Yet another aspect of the invention involves a method  
25 of producing seeds having altered fatty acid composition. The method comprises the step of introducing a recombinant nucleic acid construct into a plant (i.e., transforming a plant). The construct comprises one or more seed-specific  
30 regulatory sequences operably linked in sense orientation to a mutant delta-12 fatty acid desaturase gene or a mutant delta-15 fatty acid desaturase gene. After obtaining transgenic progeny, those transformed plants that produce seeds having an altered fatty acid composition are  
35 identified. Suitable plants for transformation include, for example, soybean, rapeseed, sunflower, safflower, castor bean and corn. Suitable methods of transforming

such plants include, for example, *Agrobacterium*- mediated methods, electroporation, and microprojectile bombardment.

The invention also is embodied in a method of RFLP breeding to obtain altered levels of oleic acids in the seed oil of oil producing plant species. This method involves (a) making a cross between two varieties of oil producing plant species differing in the oleic acid trait; (b) making a Southern blot of restriction enzyme digested genomic DNA isolated from several progeny plants resulting from the cross; and (c) hybridizing the Southern blot with the radiolabelled nucleic acid fragments encoding the mutant fatty acid desaturases or desaturase-related enzymes.

The invention is also embodied in a method of RFLP mapping that uses the isolated mutant microsomal delta-12 desaturase cDNA or related genomic fragments described herein.

Another embodiment of the instant invention is a method of genotyping plants containing either a mutant or wild-type form of the delta-12 desaturase gene by PCR amplification of genomic DNA using gene-specific primers. This method is capable of discriminating genes that differ by only one or a few nucleotides, thus affording a means for detecting plants containing the mutant delta-12 desaturase.

Another aspect of the invention comprises vegetable oil extracted from seeds of plants disclosed herein. Such a vegetable oil contains an altered fatty acid composition, e.g., a decreased level of  $\alpha$ -linolenic acid, a decreased level of linoleic acid, or an increased level of oleic acid, based on total fatty acid composition.

#### BRIEF DESCRIPTION OF THE SEQUENCE DESCRIPTIONS

The invention can be more fully understood from the following detailed description and the Sequence Descriptions which form a part of this application. The Sequence Descriptions contain the three letter codes for amino acids as defined in 37 C.F.R. 1.822 which are incorporated herein by reference.

5 SEQ ID NO:2 is the 384 amino acid protein sequence deduced from the open reading frame in SEQ ID NO:1.

SEQ ID NO:4 is the 384 amino acid protein sequence deduced from the open reading frame of SEQ ID NO:3.

SEQ ID NO:6 is the 384 amino acid protein sequence  
20 deduced from the open reading frame of SEQ ID NO:5.

SEQ ID NO:8 is the 384 amino acid protein sequence deduced from the open reading frame of SEQ ID NO:7.

SEQ ID NO:10 is the downstream (3') primer used for isolation of the D form of microsomal delta-12 fatty acid desaturase gene from *Brassica napus*.

SEQ ID NO:11 is the upstream (5') primer used for  
35 isolation of the F form of microsomal delta-12 fatty acid  
desaturase gene in *Brassica napus*.

SEQ ID NO:12 is the downstream (3') primer used for isolation of the F form of microsomal delta-12 fatty acid desaturase gene in Brassica napus.

5 SEQ ID NO:13 is the upstream (5') primer used for gene-specific detection of the wild type D form of microsomal delta-12 fatty acid desaturase gene in Brassica napus.

SEQ ID NO:14 is the upstream (5') primer used for gene-specific detection of the mutant D form of microsomal delta-12 fatty acid desaturase gene in Brassica napus.

10 SEQ ID NO:15 is the modified upstream (5') primer used for gene-specific detection of the wild type D form of microsomal delta-12 fatty acid desaturase gene in Brassica napus.

15 SEQ ID NO:16 is the modified upstream (5') primer used for gene-specific detection of the mutant D form of microsomal delta-12 fatty acid desaturase gene in Brassica napus.

#### BRIEF DESCRIPTION OF THE FIGURES

20 Figure 1 is a schematic drawing of plasmid pZPhMcFd2, showing restriction sites and relative position and orientation of the bean phaseolin promoter (5' Phas), the IMC129 mutant microsomal delta-12 fatty acid desaturase D form coding sequence (MCFd2) and the bean phaseolin 3' untranslated region (3' Phas).

25 Figure 2 is a schematic drawing of plasmid pIMC127, showing restriction sites and the relative positions and orientation of the napin promoter (5' nap), the wild-type microsomal delta-12 fatty acid desaturase D form coding sequence (CanFd2) and the napin 3' untranslated region (3' Nap).

30 Figure 3 shows the frequency distribution of seed oil linoleic acid (C18:2) content in transgenic Brassica T2 populations transformed with either a napin promoter linked in sense orientation to a wild-type microsomal delta-12 fatty acid desaturase D form coding sequence (WS127) or a phaseolin promoter linked to a mutant delta-12 fatty acid desaturase D form (WS201).

Figure 4 shows the frequency distribution of seed oil linoleic acid content in transgenic Brassica T2 populations transformed with either a napin promoter linked in sense orientation to a mutant F form (WS140) delta-12 fatty acid desaturase coding sequence or a cruciferin promoter linked to a wild-type delta-12 fatty acid desaturase D form (WS135).

#### DETAILED DESCRIPTION OF THE INVENTION

Applicants have isolated nucleic acid fragments that encode mutant plant fatty acid desaturases and that are useful in modifying fatty acid composition in oil-producing species by genetic transformation.

Transfer of the nucleic acid fragments of the invention or a part thereof, along with suitable regulatory sequences that direct the transcription of their mRNA, into plants may result in production of decreased levels of unsaturated fatty acids in cellular lipids, including triacylglycerols.

The nucleic acid fragments of the invention can also be used as DNA diagnostic markers in plant genetic mapping and plant breeding programs.

The nucleic acid fragments of the invention or oligomers derived therefrom can also be used to isolate other related fatty acid desaturase genes using DNA, RNA, or a library of cloned nucleotide sequences from the same or different species by well known sequence-dependent protocols, including, for example, methods of nucleic acid hybridization and amplification by the polymerase chain reaction.

#### Definitions

In the context of this disclosure, a number of terms shall be used. Fatty acids are specified by the number of carbon atoms and the number and position of the double bond: the numbers before and after the colon refer to the chain length and the number of double bonds, respectively. The number following the fatty acid designation indicates the position of the double bond from the carboxyl end of the fatty acid with the "c" affix for the cis-configuration of the double bond. For example, palmitic acid (16:0),

stearic acid (18:0), oleic acid (18:1,9c), petroselinic  
 acid (18:1, 6c), linoleic acid (18:2,9c,12c),  $\gamma$ -linolenic  
 acid (18:3, 6c,9c,12c) and  $\alpha$ -linolenic acid (18:3,  
 9c,12c,15c). Unless otherwise specified 18:1, 18:2 and  
 5 18:3 refer to oleic, linoleic and linolenic fatty acids.  
 The term "fatty acid desaturase" used herein refers to an  
 enzyme which catalyzes the breakage of a carbon-hydrogen  
 bond and the introduction of a carbon-carbon double bond  
 into a fatty acid molecule. The fatty acid may be free or  
 10 esterified to another molecule including, but not limited  
 to, acyl-carrier protein, coenzyme A, sterols and the  
 glycerol moiety of glycerolipids. The term "glycerolipid  
 desaturases" used herein refers to a subset of the fatty  
 acid desaturases that act on fatty acyl moieties esterified  
 15 to a glycerol backbone. "Delta-12 desaturase" refers to a  
 fatty acid desaturase that catalyzes the formation of a  
 double bond between carbon positions 6 and 7 (numbered from  
 the methyl end), (i.e., those that correspond to carbon  
 positions 12 and 13 (numbered from the carbonyl carbon) of  
 20 an 18 carbon-long fatty acyl chain. "Delta-15 desaturase"  
 refers to a fatty acid desaturase that catalyzes the  
 formation of a double bond between carbon positions 3 and 4  
 (numbered from the methyl end), (i.e., those that  
 correspond to carbon positions 15 and 16 (numbered from the  
 25 carbonyl carbon) of an 18 carbon-long fatty acyl chain.  
 Examples of fatty acid desaturases include, but are not  
 limited to, the microsomal delta-12 and delta-15  
 desaturases that act on phosphatidylcholine lipid  
 substrates; the chloroplastic or plastid delta-12 and  
 30 delta-15 desaturases that act on phosphatidyl glycerol and  
 galactolipids; and other desaturases that act on such fatty  
 acid substrates such as phospholipids, galactolipids, and  
 sulfolipids. "Microsomal desaturase" refers to the  
 cytoplasmic location of the enzyme, while "chloroplast  
 35 desaturase" and "plastid desaturase" refer to the plastid  
 location of the enzyme. These fatty acid desaturases may  
 be found in a variety of organisms including, but not  
 limited to, higher plants, diatoms, and various eukaryotic

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and prokaryotic microorganisms such as fungi and photosynthetic bacteria and algae. The term "homologous fatty acid desaturases" refers to fatty acid desaturases that catalyze the same desaturation on the same lipid substrate. Thus, microsomal delta-15 desaturases, even from different plant species, are homologous fatty acid desaturases. The term "heterologous fatty acid desaturases" refers to fatty acid desaturases that catalyze desaturations at different positions and/or on different lipid substrates. Thus, for example, microsomal delta-12 and delta-15 desaturases, which act on phosphatidylcholine lipids, are heterologous fatty acid desaturases, even when from the same plant. Similarly, microsomal delta-15 desaturase, which acts on phosphatidylcholine lipids, and chloroplast delta-15 desaturase, which acts on galactolipids, are heterologous fatty acid desaturases, even when from the same plant. It should be noted that these fatty acid desaturases have never been isolated and characterized as proteins. Accordingly, the terms such as "delta-12 desaturase" and "delta-15 desaturase" are used as a convenience to describe the proteins encoded by nucleic acid fragments that have been isolated based on the phenotypic effects caused by their disruption. They do not imply any catalytic mechanism. For example, delta-12 desaturase refers to the enzyme that catalyzes the formation of a double bond between carbons 12 and 13 of an 18 carbon fatty acid irrespective of whether it "counts" the carbons from the methyl, carboxyl end, or the first double bond.

The term "nucleic acid" refers to a large molecule which can be single-stranded or double-stranded, composed of monomers (nucleotides) containing a sugar, a phosphate and either a purine or pyrimidine. A "nucleic acid fragment" is a fraction of a given nucleic acid molecule. In higher plants, deoxyribonucleic acid (DNA) is the genetic material while ribonucleic acid (RNA) is involved in the transfer of the information in DNA into proteins. A "genome" is the entire body of genetic material contained

in each cell of an organism. The term "nucleotide sequence" refers to the sequence of DNA or RNA polymers, which can be single- or double-stranded, optionally containing synthetic, non-natural or altered nucleotide bases capable of incorporation into DNA or RNA polymers. The term "oligomer" refers to short nucleotide sequences, usually up to 100 bases long.

From time to time, the term "FAD2" may be used herein as a shorthand notation for a nucleotide sequence encoding a wild type microsomal delta-12 fatty acid desaturase enzyme, and the term "fad2" may be used herein as a shorthand notation for a nucleotide sequence encoding a mutant form of a microsomal delta-12 fatty acid desaturase enzyme.

As used herein, the term "homologous to" refers to the relatedness between the nucleotide sequence of two nucleic acid molecules or between the amino acid sequences of two protein molecules. Estimates of such homology are provided by either DNA-DNA or DNA-RNA hybridization under conditions of stringency as is well understood by those skilled in the art (Hames and Higgins, Eds. (1985) Nucleic Acid Hybridisation, IRL Press, Oxford, U.K.); or by the comparison of sequence similarity between two nucleic acids or proteins, such as by the method of Needleman et al. (J. Mol. Biol. (1970) 48:443-453). As used herein, "essentially similar" refers to DNA sequences that may involve base changes that do not cause a change in the encoded amino acid, or which involve base changes which may alter one or more amino acids, but do not affect the functional properties of the protein encoded by the DNA sequence. It is therefore understood that the invention encompasses more than the specific exemplary sequences. Modifications to the sequence, such as deletions, insertions, or substitutions in the sequence which produce silent changes that do not substantially affect the functional properties of the resulting protein molecule are also contemplated. For example, alteration in the gene sequence which reflect the degeneracy of the genetic code,



or which results in the production of a chemically equivalent amino acid at a given site, are contemplated; thus, a codon for the amino acid alanine, a hydrophobic amino acid, may be substituted by a codon encoding another hydrophobic amino acid residue such as glycine, valine, leucine, or isoleucine. Similarly, changes which result in substitution of one negatively charged residue for another, such as aspartic acid for glutamic acid, or one positively charged residue for another, such as lysine for arginine, can also be expected to produce a biologically equivalent product. Nucleotide changes which result in alteration of the N-terminal and C-terminal portions of the protein molecule would also not be expected to alter the activity of the protein. Each of the proposed modifications is well within the routine skill in the art, as is determination of retention of biological activity of the encoded products.

"Gene" refers to a nucleic acid fragment that expresses a specific protein, including regulatory sequences preceding (5' non-coding) and following (3' non-coding) the coding region. "Fatty acid desaturase gene" refers to a nucleic acid fragment that expresses a protein with fatty acid desaturase activity. "Native" gene refers to an isolated gene with its own regulatory sequences as found in nature. "Chimeric gene" refers to a gene that comprises heterogeneous regulatory and coding sequences not found in nature. "Endogenous" gene refers to the native gene normally found in its natural location in the genome and is not isolated. A "foreign" gene refers to a gene not normally found in the host organism but that is introduced by gene transfer. "Pseudo-gene" refers to a genomic nucleotide sequence that does not encode a functional enzyme. "Mutant gene" refers to a gene comprising one or more nucleotides that have been altered when compared to the wild-type nucleotide sequence, resulting in a change to the amino acid sequence and functional properties of the encoded protein.

"Coding sequence" refers to a DNA sequence that codes for a specific protein and excludes the non-coding

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sequences. It may constitute an "uninterrupted coding sequence", i.e., lacking an intron or it may include one or more introns bounded by appropriate splice junctions. An "intron" is a nucleotide sequence that is transcribed in the primary transcript but that is removed through cleavage and re-ligation of the RNA within the cell to create the mature mRNA that can be translated into a protein.

"Initiation codon" and "termination codon" refer to a unit of three adjacent nucleotides in a coding sequence that specifies initiation and chain termination, respectively, of protein synthesis (mRNA translation). "Open reading frame" refers to the coding sequence uninterrupted by introns between initiation and termination codons that encodes an amino acid sequence.

"RNA transcript" refers to the product resulting from RNA polymerase-catalyzed transcription of a DNA sequence. When the RNA transcript is a perfect complementary copy of the DNA sequence, it is referred to as the primary transcript or it may be a RNA sequence derived from posttranscriptional processing of the primary transcript and is referred to as the mature RNA. "Messenger RNA (mRNA)" refers to the RNA that is without introns and that can be translated into protein by the cell. "cDNA" refers to a double-stranded DNA that is complementary to and derived from mRNA. "Sense" RNA refers to RNA transcript that includes the mRNA. "Antisense RNA" refers to a RNA transcript that is complementary to all or part of a target primary transcript or mRNA and that blocks the expression of a target gene by interfering with the processing, transport and/or translation of its primary transcript or mRNA. The complementarity of an antisense RNA may be with any part of the specific gene transcript, i.e., at the 5' non-coding sequence, 3' non-coding sequence, introns, or the coding sequence. In addition, as used herein, antisense RNA may contain regions of ribozyme sequences that increase the efficacy of antisense RNA to block gene expression. "Ribozyme" refers to a catalytic RNA and includes sequence-specific endoribonucleases.

As used herein, "suitable regulatory sequences" refer to nucleotide sequences in native or chimeric genes that are located upstream (5'), within, and/or downstream (3') to the nucleic acid fragments of the invention, which

5 control the expression of the nucleic acid fragments of the invention. The term "expression", as used herein, refers to the transcription and stable accumulation of the sense (mRNA) derived from the nucleic acid fragment(s) of the invention that, in conjunction with the protein apparatus

10 of the cell, results in altered levels of the fatty acid desaturase(s). Expression or overexpression of the gene involves transcription of the gene and translation of the mRNA into precursor or mature fatty acid desaturase proteins. "Altered levels" refers to the production of

15 gene product(s) in transgenic organisms in amounts or proportions that differ from that of normal or non-transformed organisms.

"Promoter" refers to a DNA sequence in a gene, usually upstream (5') to its coding sequence, which controls the

20 expression of the coding sequence by providing the recognition for RNA polymerase and other factors required for proper transcription. Promoters may also contain DNA sequences that are involved in the binding of protein factors which control the effectiveness of transcription

25 initiation in response to physiological or developmental conditions. It may also contain enhancer elements. An "enhancer" is a DNA sequence which can stimulate promoter activity. It may be an innate element of the promoter or a heterologous element inserted to enhance the level and/or

30 tissue-specificity of a promoter. "Constitutive promoters" refers to those that direct gene expression in all tissues and at all times. "Tissue-specific" or "development-specific" promoters as referred to herein are those that direct gene expression almost exclusively in specific

35 tissues, such as leaves or seeds, or at specific development stages in a tissue, such as in early or late embryogenesis, respectively.

The "3' non-coding sequences" refers to the DNA sequence portion of a gene that contains a polyadenylation signal and any other regulatory signal capable of affecting mRNA processing or gene expression. The polyadenylation  
 5 signal is usually characterized by affecting the addition of polyadenylic acid tracts to the 3' end of the mRNA precursor.

"Transformation" herein refers to the transfer of a foreign gene into the genome of a host organism and its  
 10 genetically stable inheritance. "Restriction fragment length polymorphism" (RFLP) refers to different sized restriction fragment lengths due to altered nucleotide sequences in or around variant forms of genes. "Molecular breeding" refers to the use of DNA-based diagnostics, such  
 15 as RFLP, RAPDs, and PCR in breeding. "Fertile" refers to plants that are able to propagate sexually.

"Plants" refer to photosynthetic organisms, both eukaryotic and prokaryotic, whereas the term "Higher plants" refers to eukaryotic plants. "Oil-producing  
 20 species" herein refers to plant species which produce and store triacylglycerol in specific organs, primarily in seeds. Such species include soybean (Glycine max), rapeseed and canola (including Brassica napus, B. campestris), sunflower (Helianthus annus), cotton  
 25 (Gossypium hirsutum), corn (Zea mays), cocoa (Theobroma cacao), safflower (Carthamus tinctorius), oil palm (Elaeis guineensis), coconut palm (Cocos nucifera), flax (Linum usitatissimum), (castor (Ricinus communis)) and peanut  
 30 (Arachis hypogaea). The group also includes non-agronomic species which are useful in developing appropriate expression vectors such as tobacco, rapid cycling Brassica species, and Arabidopsis thaliana, and wild species which may be a source of unique fatty acids.

"Progeny" includes descendants of a particular plant or  
 35 plant line, e.g., seeds and plants of F1, F2, F3, and subsequent generations, or seeds and plants of backcrossed populations BC1, BC2, BC3 and subsequent generations.

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"Sequence-dependent protocols" refer to techniques that rely on a nucleotide sequence for their utility. Examples of sequence-dependent protocols include, but are not limited to, the methods of nucleic acid and oligomer hybridization and methods of DNA and RNA amplification such as are exemplified in various uses of the polymerase chain reaction (PCR).

Various solutions used in the experimental manipulations are referred to by their common names such as "SSC", "SSPE", "Denhardt's solution", etc. The composition of these solutions may be found by reference to Appendix B of Sambrook, et al. (Molecular Cloning, A Laboratory Manual, 2nd ed. (1989), Cold Spring Harbor Laboratory Press).

#### 15      AVAILABILITY AND RELATEDNESS OF WILD-TYPE MICROSOMAL          DELTA-12 AND DELTA-15 FATTY ACID DESATURASES

United States Patent Application No. 08/262,401, incorporated herein by reference, describes the isolation and characterization of cDNAs encoding wild-type microsomal delta-12 fatty acid desaturases from a number of plant species, including Arabidopsis thaliana, Brassica napus, Glycine max, Zea mays and Castor bean. Moreover, that application demonstrates successful alteration of fatty acid content of oils from seeds obtained from transgenic plants expressing sense or antisense mRNAs encoding microsomal delta-12 fatty acid desaturases.

Alignments of protein sequences of plant microsomal fatty acid delta-12 desaturases and plant delta-15 desaturases [microsomal and plastid delta-15 desaturases from Arabidopsis and Brassica napus, WO 9311245] allows identification of amino acid sequences conserved between the different desaturases (Table 1).

TABLE 1  
Amino Acid Sequences Conserved Between  
Plant Microsomal Delta-12 Desaturases and Microsomal and  
Plastid Delta-15 Desaturases

Region	Conserved AA Positions in SEQ ID NO:2 of USSN 08/262,401	Consensus Conserved AA Sequence in $\Delta^{12}$ Desaturases	Consensus Conserved AA Sequence in $\Delta^{15}$ Desaturases	Consensus AA Sequence
A	39-44	AIPPHC	AIPKHC	AIP(P/K)HC
B	86-90	WP(L/I)YW	WPLYW	WP(L/I)YW
C	104-109	AHECGH	GHDCGH	(A/G)H(D/E)CGH
D	130-134	LLVPY	ILVPY	(L/I)LVPY
E	137-142	WKYSHR	WRISHR	W(K/R)(Y/I)SHR
F	140-145	SHRRHH	SHRTHH	SHR(R/T)HH
G	269-274	ITYLQ	VTYLH	(I/V)TYL(Q/H)
H	279-282	LPHY	LPWY	LP(H/W)Y
I	289-294	WL(R/K)GAL	YLRGGL	(W/Y)L(R/K)G(A/G)L
J	296-302	TVDRDYG	TLDRDYG	T(V/L)DRDYG
K	314-321	THVAHHLF	THVIHHLF	THV(A/I)HHLF
L	318-327	HHLFSTMPHY	HHLFPQIPHY	HHLF(S/P) (T/Q)(I/M)PHY

Table 1 shows twelve regions of conserved amino acid sequences, designated A-L (column 1), whose positions in SEQ ID NO:2 of USSN 08/262,401 are shown in column 2. The consensus sequences for these regions in plant delta-12 fatty acid desaturases and plant delta-15 fatty acid desaturases are shown in columns 3 and 4, respectively; amino acids are shown by standard abbreviations, the underlined amino acids are conserved between the delta-12 and the delta-15 desaturases, and amino acids in brackets represent substitutions found at that position. The consensus sequence of these regions are shown in column 5. These short conserved amino acids and their relative positions further confirm that the isolated cDNAs encode a fatty acid desaturase.

#### INHIBITION OF PLANT TARGET GENES BY DOMINANT NEGATIVE SUPPRESSION

In one embodiment, transgenic plants according to the invention contain an introduced nucleic acid construct that comprises at least a portion of a mutant delta-12 or

delta-15 desaturase coding sequence. Surprisingly, a construct comprising a mutant delta-12 desaturase or delta-15 desaturase coding sequence, operably linked in sense orientation to one or more regulatory sequences, has  
5 been found to inhibit the corresponding endogenous fatty acid desaturase activity in plants which contain such a construct. This phenomenon has been termed dominant negative suppression.

The basis for the phenomenon of dominant negative  
10 suppression is not understood. One possible explanation is that the delta-12 desaturase gene product exists as a dimer *in vivo*. If so, a dimer consisting of the mutant gene product and the wild-type gene product may be non-functional. Regardless of the actual mechanism by which  
15 dominant negative suppression operates, the observation that transformation of plants with a mutant delta-12 desaturase gene results in a large proportion of the transgenic progeny having endogenous wild-type enzyme activity inhibited by expression of the enzyme gene can be  
20 used to advantage. For example, the phenomenon of dominant negative suppression can be used to alter plant desaturase enzyme activity in a tissue-specific manner. The phenomenon may also allow transformation experiments to be carried out in which a higher proportion of the resulting  
25 transgenic plants have a desired altered fatty acid profile and allow transgenic plants having desired fatty acid profiles to be more readily obtained.

Preferred constructs contain, in addition, at least one regulatory sequence operably linked in the sense  
30 orientation to the mutant coding sequence. Regulatory sequences typically do not themselves code for a gene product. Instead, regulatory sequences affect the expression level of the mutant coding sequence.

In preferred embodiments, regulatory sequences for  
35 dominant negative suppression are tissue-specific, i.e., the mutant desaturase gene product is preferentially expressed in certain plant tissues and expressed at low levels or not at all in the remaining tissues of the plant.

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about 10%  $\alpha$ -linolenic acid, preferably from about <1% to about 5%, based on total seed fatty acid composition.

In one embodiment of the invention, a plant contains a mutant delta-12 fatty acid desaturase and a mutant delta-15 fatty acid desaturase, both of which are expressed preferentially in seeds. Such a plant can be produced from the cross of single mutant plants, followed by outcrossing or selfing in order to obtain progeny seeds carrying both mutant chimeric genes. Progeny seeds are screened in order to identify those seeds carrying both mutant genes. Alternatively, seed-specific defects in delta-12 desaturase and delta-15 desaturase may be introduced into a wild-type plant by transformation, simultaneously or sequentially, with one or more dominant negative suppression constructs for delta-12 desaturase and delta-15 desaturase, each driven by suitable regulatory sequences. Other similar methods to construct double mutant plants will be recognized by those of skill in the art.

Double mutant plants can have more extreme fatty acid profiles in seeds than the single mutant plants, e.g., the double mutant phenotype can result in significantly lower levels of  $\alpha$ -linolenic acid in seeds than the single mutant delta-15 desaturase plant phenotype. Thus, by combining seed-specific inhibition of microsomal delta-12 desaturase with seed-specific inhibition of microsomal delta-15 desaturase, one can obtain levels of seed  $\alpha$ -linolenic acid that are as low or lower than those in a single mutant without adversely affecting desirable properties. The decreased levels of  $\alpha$ -linolenic acid in the double homozygotes may be associated with increased levels of oleic acid and decreased levels of saturates and linoleic acid.

#### SELECTION OF HOSTS, PROMOTERS AND ENHANCERS

A preferred class of heterologous hosts for the expression of the nucleic acid fragments of the invention are eukaryotic hosts, particularly the cells of higher plants. Particularly preferred among the higher plants are the oil-producing species, such as soybean (Glycine max),

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storage proteins are strictly regulated, being expressed almost exclusively in seeds in a highly tissue-specific and stage-specific manner (Higgins et al., *Ann. Rev. Plant Physiol.* (1984) 35:191-221; Goldberg et al., *Cell* (1989) 56:149-160). Moreover, different seed storage proteins may be expressed at different stages of seed development.

Expression of seed-specific genes has been studied in great detail (See reviews by Goldberg et al., *Cell* (1989) 56:149-160 and Higgins et al., *Ann. Rev. Plant Physiol.* (1984) 35:191-221). There are currently numerous examples of seed-specific expression of seed storage protein genes in transgenic dicotyledonous plants. These include genes from dicotyledonous plants for bean  $\beta$ -phaseolin (Sengupta-Gopalan et al., *Proc. Natl. Acad. Sci. USA* (1985) 82:3320-3324; Hoffman et al., *Plant Mol. Biol.* (1988) 11:717-729), bean lectin (Voelker et al., *EMBO J.* (1987) 6:3571-3577), soybean lectin (Okamuro et al., *Proc. Natl. Acad. Sci. USA* (1986) 83:8240-8244), soybean Kunitz trypsin inhibitor (Perez-Grau et al., *Plant Cell* (1989) 1:095-1109), soybean  $\beta$ -conglycinin (Beachy et al., *EMBO J.* (1985) 4:3047-3053; pea vicilin (Higgins et al., *Plant Mol. Biol.* (1988) 11:683-695), pea convicilin (Newbigin et al., *Planta* (1990) 180:461-470), pea legumin (Shirsat et al., *Mol. Gen. Genetics* (1989) 215:326-331); rapeseed napin (Radke et al., *Theor. Appl. Genet.* (1988) 75:685-694) as well as genes from monocotyledonous plants such as for maize 15 kD zein (Hoffman et al., *EMBO J.* (1987) 6:3213-3221), maize 18 kD oleosin (Lee et al., *Proc. Natl. Acad. Sci. USA* (1991) 88:6181-6185), barley  $\beta$ -hordein (Marris et al., *Plant Mol. Biol.* (1988) 10:359-366) and wheat glutenin (Colot et al., *EMBO J.* (1987) 6:3559-3564). Moreover, promoters of seed-specific genes operably linked to heterologous coding sequences in chimeric gene constructs also maintain their temporal and spatial expression pattern in transgenic plants. Such examples include use of *Arabidopsis thaliana* 2S seed storage protein gene promoter to express enkephalin peptides in *Arabidopsis* and *B. napus* seeds (Vandekerckhove et al., *Bio/Technology*

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(1989) 7:929-932), bean lectin and bean  $\beta$ -phaseolin promoters to express luciferase (Riggs et al., *Plant Sci.* (1989) 63:47-57), and wheat glutenin promoters to express chloramphenicol acetyl transferase (Colot et al., *EMBO J.* (1987) 6:3559-3564).

Of particular use in the expression of the nucleic acid fragment of the invention will be the heterologous promoters from several soybean seed storage protein genes such as those for the Kunitz trypsin inhibitor (Jofuku et al., *Plant Cell* (1989) 1:1079-1093; glycinin (Nielson et al., *Plant Cell* (1989) 1:313-328), and  $\beta$ -conglycinin (Harada et al., *Plant Cell* (1989) 1:415-425). Promoters of genes for  $\alpha$ - and  $\beta$ -subunits of soybean  $\beta$ -conglycinin storage protein will be particularly useful in expressing the mRNA or the antisense RNA in the cotyledons at mid- to late-stages of seed development (Beachy et al., *EMBO J.* (1985) 4:3047-3053) in transgenic plants. This is because there is very little position effect on their expression in transgenic seeds, and the two promoters show different temporal regulation. The promoter for the  $\alpha$ -subunit gene is expressed a few days before that for the  $\beta$ -subunit gene. This is important for transforming rapeseed where oil biosynthesis begins about a week before seed storage protein synthesis (Murphy et al., *J. Plant Physiol.* (1989) 135:63-69).

Also of particular use will be promoters of genes expressed during early embryogenesis and oil biosynthesis. The native regulatory sequences, including the native promoters, of the fatty acid desaturase genes expressing the nucleic acid fragments of the invention can be used following their isolation by those skilled in the art. Heterologous promoters from other genes involved in seed oil biosynthesis, such as those for *B. napus* isocitrate lyase and malate synthase (Comai et al., *Plant Cell* (1989) 1:293-300), delta-9 desaturase from safflower (Thompson et al. *Proc. Natl. Acad. Sci. USA* (1991) 88:2578-2582) and castor (Shanklin et al., *Proc. Natl. Acad. Sci. USA* (1991) 88:2510-2514), acyl carrier protein (ACP) from *Arabidopsis*

(Post-Beittenmiller et al., *Nucl. Acids Res.* (1989) 17:1777), B. napus (Safford et al., *Eur. J. Biochem.* (1988) 174:287-295), and B. campestris (Rose et al., *Nucl. Acids Res.* (1987) 15:7197),  $\beta$ -ketoacyl-ACP synthetase from barley (Siggaard-Andersen et al., *Proc. Natl. Acad. Sci. USA* (1991) 88:4114-4118), and oleosin from Zea mays (Lee et al., *Proc. Natl. Acad. Sci. USA* (1991) 88:6181-6185), soybean (Genbank Accession No: X60773) and B. napus (Lee et al., *Plant Physiol.* (1991) 96:1395-1397) will be of use.

If the sequence of the corresponding genes is not disclosed or their promoter region is not identified, one skilled in the art can use the published sequence to isolate the corresponding gene and a fragment thereof containing the promoter. The partial protein sequences for the relatively-abundant enoyl-ACP reductase and acetyl-CoA carboxylase are also published (Slabas et al., *Biochim. Biophys. Acta* (1987) 877:271-280; Cottingham et al., *Biochim. Biophys. Acta* (1988) 954:201-207) and one skilled in the art can use these sequences to isolate the corresponding seed genes with their promoters. Similarly, the fragments of the present invention encoding fatty acid desaturases can be used to obtain promoter regions of the corresponding genes for use in expressing chimeric genes.

Attaining the proper level of expression of the nucleic acid fragments of the invention may require the use of different chimeric genes utilizing different promoters. Such chimeric genes can be transferred into host plants either together in a single expression vector or sequentially using more than one vector.

It is envisioned that the introduction of enhancers or enhancer-like elements into the promoter regions of either the native or chimeric nucleic acid fragments of the invention will result in increased expression to accomplish the invention. This would include viral enhancers such as that found in the 35S promoter (Odell et al., *Plant Mol. Biol.* (1988) 10:263-272), enhancers from the opine genes (Fromm et al., *Plant Cell* (1989) 1:977-984), or enhancers from any other source that result in increased

transcription when placed into a promoter operably linked to a nucleic acid fragment of the invention.

Of particular importance is the DNA sequence element isolated from the gene for the  $\alpha$ -subunit of  $\beta$ -conglycinin that can confer 40-fold seed-specific enhancement to a constitutive promoter (Chen et al., *Dev. Genet.* (1989) 10:112-122). One skilled in the art can readily isolate this element and insert it within the promoter region of any gene in order to obtain seed-specific enhanced expression with the promoter in transgenic plants. Insertion of such an element in any seed-specific gene that is expressed at different times than the  $\beta$ -conglycinin gene will result in expression in transgenic plants for a longer period during seed development.

The invention can also be accomplished by a variety of other methods to obtain the desired end. In one form, the invention is based on modifying plants to produce increased levels of mutant fatty acid desaturases by virtue of introducing more than one copy of the foreign gene containing the nucleic acid fragments of the invention. In some cases, the desired level of polyunsaturated fatty acids may require introduction of foreign genes for more than one kind of mutant fatty acid desaturase.

Any 3' non-coding region capable of providing a polyadenylation signal and other regulatory sequences that may be required for the proper expression of the nucleic acid fragments of the invention can be used to accomplish the invention. This would include 3' ends of the native fatty acid desaturase(s), viral genes such as from the 35S or the 19S cauliflower mosaic virus transcripts, from the opine synthesis genes, ribulose 1,5-bisphosphate carboxylase, or chlorophyll a/b binding protein. There are numerous examples in the art that teach the usefulness of different 3' non-coding regions.

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#### TRANSFORMATION METHODS

Various methods of transforming cells of higher plants according to the present invention are available to those skilled in the art (see EPO Pub. 0 295 959 A2 and

0 318 341 A1). Such methods include those based on transformation vectors utilizing the Ti and Ri plasmids of Agrobacterium spp. It is particularly preferred to use the binary type of these vectors. Ti-derived vectors transform a wide variety of higher plants, including monocotyledonous and dicotyledonous plants (Sukhapinda et al., *Plant Mol. Biol.* (1987) 8:209-216; Potrykus, *Mol. Gen. Genet.* (1985) 199:183). Other transformation methods are available to those skilled in the art, such as direct uptake of foreign DNA constructs (see EPO Pub. 0 295 959 A2), techniques of electroporation (Fromm et al., *Nature* (1986) (London) 319:791) or high-velocity ballistic bombardment with metal particles coated with the nucleic acid constructs (Kline et al., *Nature* (1987) (London) 327:70). Once transformed, the cells can be regenerated by those skilled in the art.

Of particular relevance are the recently described methods to transform foreign genes into commercially important crops, such as rapeseed (De Block et al., *Plant Physiol.* (1989) 91:694-701), sunflower (Everett et al., *Bio/Technology* (1987) 5:1201), and soybean (Christou et al., *Proc. Natl. Acad. Sci USA* (1989) 86:7500-7504).

#### APPLICATION TO PLANT BREEDING

The use of restriction fragment length polymorphism (RFLP) markers in plant breeding has been well-documented in the art (Tanksley et al., *Bio/Technology* (1989) 7:257-264). Thus, the nucleic acid fragments of the invention can be used as molecular markers for traits associated with mutant fatty acid desaturases. These traits will include altered levels of unsaturated fatty acids. The nucleic acid fragment of the invention can also be used to isolate the fatty acid desaturase gene from other mutant plants with altered levels of unsaturated fatty acids. Sequencing of these genes will reveal nucleotide differences that cause the alteration in levels of unsaturated fatty acids. Oligonucleotides designed around these differences may also be used in plant breeding as diagnostic markers to follow fatty acid variation. In one embodiment, oligonucleotides based on differences

between wt and mutant  $\Delta 12$  des may be used as molecular markers in breeding canola lines with variant oil profiles.

#### EXAMPLES

The present invention is further defined in the following Examples, in which all parts and percentages are by weight and degrees are Celsius, unless otherwise stated. It should be understood that these Examples, while indicating preferred embodiments of the invention, are given by way of illustration only. From the above discussion and these Examples, one skilled in the art can ascertain the essential characteristics of this invention, and without departing from the spirit and scope thereof, can make various changes and modifications of the invention to adapt it to various usages and conditions. All publications, including patents and non-patent literature, referred to in this specification are expressly incorporated by reference herein.

#### EXAMPLE 1

#### SEQUENCES OF MUTANT DELTA-12 FATTY ACID

#### DESATURASES FROM B. NAPUS

Primers specific for the FAD2 structural gene were used to clone the entire open reading frame (ORF) of the D and F forms of the gene by reverse transcription-polymerase chain reaction (RT-PCR). The sequences of the primers used for isolation of the D form ORF of B. napus FAD2 gene are

5'-CATGGGTGCAGGTGGAAGAATGC-3' (SEQ ID NO: 9); and

5'-GTTTCTTCTTTGCTTCATAAC-3' (SEQ ID NO: 10).

The sequences of the primers used to clone the F form ORF of B. napus FAD2 gene are

5'-CATGGGTGCAGGTGGAAGAATGC-3' (SEQ ID NO: 11); and

5'-TCTTTCACCATCATCATATCC-3' (SEQ ID NO: 12).

RNA from seeds of three lines, IMC129, Q508 and Westar, was isolated by an acid guanidinium thiocyanate-phenol-chloroform extraction method (Chomczynski and Sacchi, 1987; *Analytical Biochemistry* 162, 156-159, 1987). The total RNA was used as a template for reverse transcription and PCR



amplification by RNA PCR kit (Perkin Elmer). The RT-PCR amplified fragments were cloned into pGEM-T vector (Promega), and then used for nucleotide sequence determination. The DNA sequence of each gene from each  
 5 line was determined from both strands by dideoxy sequencing by Sanger et al. (Proc Natl Acad Sci USA 74, 5463-5467).

The D gene of IMC129 contained a G to A transversion at nucleotide 316 (from the translation initiation codon) of the D gene in IMC129, compared to the sequence of Westar.  
 10 The transversion changes the codon at this position from GAG to AAG and results in a substitution of glutamic acid for lysine. The same base change was also detected in IMC129 when RNA from leaf tissue was used as template. The G to A mutation at nucleotide 316 was confirmed by  
 15 sequencing several independent clones containing fragments amplified directly from genomic DNA of IMC129. These results eliminated the possibility of a rare mutation introduced during reverse transcription and PCR in the RT-PCR protocol. The mutation in the D form of delta-12  
 20 desaturase in IMC129 mapped to a conserved region of cloned delta-12 and delta-15 membrane bound-desaturases (Table 5).

The sequence of the F form of delta-12 desaturase in IMC129 was the same as the F form of delta-12 desaturase in Westar.

25 For Q508, the sequence of the D form of delta-12 desaturase was the same as the D form of the IMC129 gene. This was expected, as Q508 was derived by mutagenesis of IMC129.

Sequence analysis of the Q508 F form of delta-12  
 30 desaturase revealed a T to A transition at nucleotide 515, compared to the wild-type Westar sequence. This mutation results in a change from a CTC codon to a CAC codon, substituting a histidine residue for the wild-type leucine residue.

TABLE 2

Alignment of Amino Acid Sequences of Cloned Canola  
Membrane Bound-Desaturases

Desaturase Gene	Sequence <sup>a</sup>	Position <sup>b</sup>
Canola-FAD2-D	HECGH	110
Canola-FAD2-F	HECGH	110
Canola FAD6 <sup>c</sup>	HDCAH	171
Canola-FAD3 <sup>d</sup>	HDCGH	97
<u>Canola-FAD7<sup>e</sup></u>	HDCGH	126

<sup>a</sup>One letter amino acid code; conservative substitutions  
are underlined

<sup>b</sup>Position in gene product of first amino acid

<sup>c</sup>FAD6 = Plastid delta-12

<sup>d</sup>FAD3 = Microsomal delta-15

<sup>e</sup>FAD7 = Plastid delta-15

## EXAMPLE 2

GENE-SPECIFIC OLIGONUCLEOTIDE MARKERS FOR THE  
MUTANT AND WILD TYPE DELTA-12 FATTY ACID DESATURASE GENES

The D form of IMC129 fad2 gene contains a G to A  
transversion at nucleotide 316 from the translation  
initiation codon. Two short oligonucleotide upstream (5')  
primers, based on the single base change (G to A) between  
the D form of the IMC129 and wild type FAD2 genes, were  
designed. The sequences of the upstream (5') primers are  
as follows:

5' gene-specific primer for wild type FAD2-D:

5'-GTCTGGGTCATAGCCCACG-3' (SEQ ID NO:13); and

5' gene-specific primer for IMC129 fad2-d:

5'-GTCTGGGTCATAGCCCACA-3' (SEQ ID NO:14).

A common downstream (3') primer (SEQ ID NO: 10) specific  
for the D form of the FAD2 gene was used for both IMC129  
and wild type FAD2 genes. These gene-specific primers were  
used in a DNA based PCR diagnostic assay to genotype plants  
carrying the mutant and/or wild type FAD2 genes.

Genomic DNA was isolated from leaf tissue of IMC129 and  
Westar plants, and used as PCR templates. The PCR  
amplification assays were carried out by using a gene  
amplification kit (Perkin Elmer). The PCR conditions are  
as follows: denaturing temperature, 95°C for 1 min;

annealing temperature, 52°C for 1 min; amplification temperature 72°C for 1 min. Total 20 PCR cycles were extended. Under these conditions primers SEQ ID NO:13 and SEQ ID NO:14 only amplified wild type FAD2-D and IMC 129 mutant fad2-d gene fragments, respectively.

The specificity of the gene-specific primers could be further improved by shortening the length of the primers and by replacing the base C with a T at the second position from the 3' end of the oligonucleotide PCR primer for FAD2-D (SEQ ID NO:13). The sequences of the modified upstream (5') oligonucleotide PCR primers are as follows:

5' modified gene-specific primer for wild type FAD2-D:  
 5'-CTGGGTCATAGCCCATG-3' (SEQ ID NO:15); and  
 5' modified gene-specific primer for IMC129 fad2-d:  
 5'-CTGGGTCATAGCCCACA-3' (SEQ ID NO:16).

The same common downstream (3') oligonucleotide primer (SEQ ID NO:10) was used for amplifying FAD2-D and fad2-d. With the modified primers, the genotype for FAD2-D and fad2-d could be consistently distinguished after extended 30 cycle of PCR amplification. Therefore, the DNA based PCR assay provided a simple and reliable method of genotyping B. Napus germplasms containing mutant and/or wild type FAD2 genes.

### EXAMPLE 3

#### CONSTRUCTS FOR DOMINANT NEGATIVE SUPPRESSION OF DELTA-12 FATTY ACID DESATURASE

The vector pZS212 was used to construct plasmids for dominant negative suppression experiments. One construct was prepared by inserting the full-length mutant D gene coding sequence (nucleotides 1 to 1155 of SEQ ID NO:3) in sense orientation between the phaseolin promoter and phaseolin 3' poly A region of plasmid pCW108. The pCW108 vector contains the bean phaseolin promoter and 3' untranslated region and was derived from the commercially available pUC18 plasmid (Gibco-BRL) via plasmids AS3 and pCW104. Plasmid AS3 contains 495 base pairs of the bean

(*Phaseolus vulgaris*) phaseolin (7S seed storage protein) promoter starting with 5'-TGGTCTTTTGGT-3' followed by the entire 1175 base pairs of the 3' untranslated region of the same gene (see sequence descriptions in Doyle et al., (1986) *J. Biol. Chem.* 261:9228-9238 and Slightom et al., (1983) *Proc. Natl. Acad. Sci. USA*, 80:1897-1901. Further sequence description may be found in WO 9113993) cloned into the Hind III site of pUC18. The additional cloning sites of the pUC18 multiple cloning region (Eco RI, Sph I, Pst I and Sal I) were removed by digesting with Eco RI and Sal I, filling in the ends with Klenow and religating to yield the plasmid pCW104. A new multiple cloning site was created between the 495bp of the 5' phaseolin and the 1175bp of the 3' phaseolin by inserting a dimer of complementary synthetic oligonucleotides consisting of the coding sequence for a Nco I site (5'-CCATGG-3') followed by three filler bases (5'-TAG-3'), the coding sequence for a Sma I site (5'-CCCGGG-3'), the last three bases of a Kpn I site (5'-TAC-3'), a cytosine and the coding sequence for an Xba I site (5'-TCTAGA-3') to create the plasmid pCW108. This plasmid contains unique Nco I, Sma I, Kpn I and Xba I sites directly behind the phaseolin promoter.

The resulting 5'-phaseolin promoter-mutant fad2-phaseolin poly A-3' construct was excised and cloned between the EcoRI/SalI sites of pZS212, resulting in the plasmid designated pZPhMCFd2 (Figure 1). pZS212 is based on a vector which contains: (1) the chimeric gene nopaline synthase/neomycin phosphotransferase as a selectable marker for transformed plant cells (Brevan et al. (1984) *Nature* 304: 184-186), (2) the left and right borders of the T-DNA of the Ti plasmid (Brevan et al. (1984) *Nucl. Acids Res.* 12:8711-8720), (3) the *E. coli* lacZ  $\alpha$ -complementing segment (Vieria and Messing (1982) *Gene* 19:259-267) with unique restriction endonuclease sites for Eco RI, Kpn I, Bam HI, and Sal I, (4) the bacterial replication origin from the *Pseudomonas* plasmid pVS1 (Itoh et al. (1984) *Plasmid* 11:206-220), and (5) the bacterial neomycin

phosphotransferase gene from Tn5 (Berg et al. (1975) *Proc. Natnl. Acad. Sci. U.S.A.* 72:3628-3632) as a selectable marker for transformed A. tumefaciens. The nopaline synthase promoter in the plant selectable marker was replaced by the 35S promoter (Odell et al. (1985) *Nature*, 313:810-813) by a standard restriction endonuclease digestion and ligation strategy.

A second plasmid was constructed by inserting the full-length wild type canola D gene coding sequence (nucleotides 130 to 1281 of SEQ ID NO:1) into the NotI site of the canola napin promoter expression vector pIMC401 which contains a 2.2 kb napin expression cassette.

The canola napin promoter expression cassette in pIMC401 was constructed as follows: ten oligonucleotide primers were synthesized based upon the nucleotide sequence of napin lambda clone CGN1-2 published in European Patent Application EP 255378). The oligonucleotide sequences were:

- BR42 and BR43 corresponding to bases 1132 to 1156 (BR42) and the complement of bases 2248 to 2271 (BR43) of the sequence listed in Figure 2 of EP 255378.
- BR45 and BR46 corresponding to bases 1150 to 1170 (BR46) and the complement of bases 2120 to 2155 (BR45) of the sequence listed in Figure 2 of EP 255378. In addition BR46 had bases corresponding to a Sal I site (5'-GTCGAC-3') and a few additional bases (5'-TCAGGCCT-3') at its 5' end and BR45 had bases corresponding to a Bgl II site (5'-AGATCT-3') and two (5'-CT-3') additional bases at the 5' end of the primer,
- BR47 and BR48 corresponding to bases 2705 to 2723 (BR47) and bases 2643 to 2666 (BR48) of the sequence listed in Figure 2 of EP 255378. In addition BR47 had two (5'-CT-3') additional bases at the 5' end of the primer followed by bases corresponding to a Bgl II site (5'-AGATCT-3') followed by a few additional bases (5'-TCAGGCCT-3'),
- BR49 and BR50 corresponding to the complement of bases 3877 to 3897 (BR49) and the complement of bases 3985 to

3919 (BR50) of the sequence listed in Figure 2 of EP 255378. In addition BR49 had bases corresponding to a Sal I site (5'-GTCGAC-3') and a few additional bases (5'-TCAGGCCT-3') at its 5' end,

- 5 • BR57 and BR58 corresponding to the complement of bases 3875 to 3888 (BR57) and bases 2700 to 2714 (BR58) of the sequence listed in Figure 2 of EP 255378. In addition the 5' end of BR57 had some extra bases (5'-CCATGG-3') followed by bases corresponding to a  
10 Sac I site (5'-GAGCTC-3') followed by more additional bases (5'-GTCGACGAGG-3'). The 5' end of BR58 had additional bases (5'-GAGCTC-3') followed by bases corresponding to a Nco I site (5'-CCATGG-3') followed by additional bases (5'-AGATCTGGTACC-3').
- 15 • BR61 and BR62 corresponding to bases 1846 to 1865 (BR61) and bases 2094 to 2114 (BR62) of the sequence listed in Figure 2 of EP 255378. In addition the 5' end of BR 62 had additional bases (5'-GACA-3') followed by bases corresponding to a Bgl II site (5'-AGATCT-3') followed  
20 by a few additional bases (5'-GCGGCCGC-3').

Genomic DNA from the canola variety 'Hyola401' (Zeneca Seeds) was used as a template for PCR amplification of the napin promoter and napin terminator regions. The promoter was first amplified using primers BR42 and BR43, and  
25 reamplified using primers BR45 and BR46. Plasmid pIMC01 was derived by digestion of the 1.0 kb promoter PCR product with SalI/BglIII and ligation into SalI/BamHI digested pBluescript SK<sup>+</sup> (Stratagene). The napin terminator region was amplified using primers BR48 and BR50, and reamplified  
30 using primers BR47 and BR49. Plasmid pIMC06 was derived by digestion of the 1.2 kb terminator PCR product with SalI/BglIII and ligation into SalI/BglIII digested pSP72 (Promega). Using pIMC06 as a template, the terminator region was reamplified by PCR using primer BR57 and primer  
35 BR58. Plasmid pIMC101 containing both the napin promoter and terminator was generated by digestion of the PCR product with SacI/NcoI and ligation into SacI/NcoI digested pIMC01. Plasmid pIMC101 contains a 2.2 kb napin expression

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cassette including complete napin 5' and 3' non-translated sequences and an introduced NcoI site at the translation start ATG. Primer BR61 and primer BR62 were used to PCR amplify an ~270 bp fragment from the 3' end of the napin promoter. Plasmid pIMC401 was obtained by digestion of the resultant PCR product with EcoRI/BglII and ligation into EcoRI/BglII digested pIMC101. Plasmid pIMC401 contains a 2.2 kb napin expression cassette lacking the napin 5' non-translated sequence and includes a NotI site at the transcription start.

The fragment containing the 5'-napin-fad2D-napin poly A-3' cassette was then inserted into the SalI site of pZS212, and the resulting 17.2 Kb plasmid was termed pIMC127 (Figure 2).

A third plasmid, pIMC135, was constructed in a manner similar to that described above for pIMC127. Plasmid pIMC135 contains a 5' cruciferin promoter fragment operably linked in sense orientation to the full-length wild-type coding sequence of SEQ ID NO:1, followed by a cruciferin 3' poly A fragment.

A fourth plasmid, pIMC140 was constructed in a manner similar to that described above. Plasmid pIMC140 contains a 5' napin promoter fragment operably linked in sense orientation to the full-length mutant Q508 F gene coding sequence (SEQ ID NO:7), followed by a 3' napin poly A fragment.

#### EXAMPLE 4

##### FATTY ACID PROFILES IN DOMINANT NEGATIVE SUPPRESSION PLANTS

The plasmids pZPhMCFd2, pIMC127, pIMC135 and pIMC140 were transferred by a freeze/thaw method (Holsters et al. (1978) *Mol Gen Genet* 163:181-187) to the Agrobacterium strain LBA4404/pAL4404 (Hockema et al. (1983), *Nature* 303:179-180).

Brassica napus cultivar "Westar" was transformed by co-cultivation of seedling pieces with disarmed Agrobacterium tumefaciens strain LBA4404 carrying the appropriate binary vector.

B. napus seeds were sterilized by stirring in 10% Chlorox, 0.1% SDS for thirty min, and then rinsed thoroughly with sterile distilled water. The seeds were germinated on sterile medium containing 30 mM CaCl<sub>2</sub> and 1.5% agar, and grown for six days in the dark at 24°C.

Liquid cultures of Agrobacterium for plant transformation were grown overnight at 28°C in Minimal A medium containing 100 mg/L kanamycin.

#### 10 Minimal A Bacterial Growth Medium

Dissolve in distilled water:

10.5 grams potassium phosphate, dibasic

4.5 grams potassium phosphate, monobasic

1.0 gram ammonium sulfate

15 0.5 gram sodium citrate, dihydrate

Make up to 979 mL with distilled water

Autoclave

Add 20 mL filter-sterilized 10% sucrose

Add 1 mL filter-sterilized 1 M MgSO<sub>4</sub>

20

The bacterial cells were pelleted by centrifugation and resuspended at a concentration of 10<sup>8</sup> cells/mL in liquid Murashige and Skoog Minimal Organic medium (GIBCO; Cat. No. 510-3118) containing 100 µM acetosyringone.

25 B. napus seedling hypocotyls were cut into 5 mm segments which were immediately placed into the bacterial suspension. After 30 min, the hypocotyl pieces were removed from the bacterial suspension and placed onto BC-35 callus medium containing 100 µM acetosyringone.

30

#### Brassica Callus Medium BC-35

Per liter:

Murashige and Skoog Minimal Organic Medium (MS salts, 100 mg/L i-inositol, 0.4 mg/L thiamine; GIBCO #510-3118)

35

30 grams sucrose

18 grams mannitol

0.5 mg/L 2,4-D

0.3 mg/L kinetin

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0.6% agarose

pH 5.8

The plant tissue and Agrobacteria were co-cultivated for  
5 three days at 24°C in dim light.

The co-cultivation was terminated by transferring the  
hypocotyl pieces to BC-35 callus medium containing 200 mg/L  
carbenicillin to kill the Agrobacteria, and 25 mg/L  
kanamycin to select for transformed plant cell growth. The  
10 seedling pieces were incubated on this medium for three  
weeks at 28°C under continuous light.

After four weeks, the segments were transferred to  
BS-48 regeneration medium containing 200 mg/L carbenicillin  
and 25 mg/L kanamycin.  
15

Brassica Regeneration Medium BS-48

Murashige and Skoog Minimal Organic Medium  
Gamborg B5 Vitamins (SIGMA #1019)  
10 grams glucose  
20 250 mg xylose  
600 mg MES  
0.4% agarose  
pH 5.7

Filter-sterilize and add after autoclaving:  
25 2.0 mg/L zeatin  
0.1 mg/L IAA

Plant tissue was subcultured every two weeks onto fresh  
selective regeneration medium, under the same culture  
30 conditions described for the callus medium. Putatively  
transformed calli grew rapidly on regeneration medium; as  
calli reached a diameter of about 2 mm, they were removed  
from the hypocotyl pieces and placed on the same medium  
lacking kanamycin.

35 Shoots began to appear within several weeks after  
transfer to BS-48 regeneration medium. As soon as the  
shoots formed discernable stems, they were excised from the  
calli, transferred to MSV-1A elongation medium, and moved  
to a 16:8 h photoperiod at 24°C.  
40

SEQUENCE LISTING

## (1) GENERAL INFORMATION:

- (i) APPLICANT: MIAO, GUO-HUA
- (ii) TITLE OF INVENTION: GENES FOR MUTANT MICROSOMAL  
FATTY ACID DELTA-12  
DESATURASES AND RELATED  
ENZYMES FROM PLANTS
- (iii) NUMBER OF SEQUENCES: 16
- (iv) CORRESPONDENCE ADDRESS:  
(A) ADDRESSEE: E. I. DU PONT DE NEMOURS  
AND COMPANY  
(B) STREET: 1007 MARKET STREET  
(C) CITY: WILMINGTON  
(D) STATE: DELAWARE  
(E) COUNTRY: U.S.A.  
(F) ZIP: 19898
- (v) COMPUTER READABLE FORM:  
(A) MEDIUM TYPE: FLOPPY DISK  
(B) COMPUTER: IBM PC COMPATIBLE  
(C) OPERATING SYSTEM: MICROSOFT WINDOWS 3.1  
(D) SOFTWARE: MICROSOFT WORD 6.0
- (vi) CURRENT APPLICATION DATA:  
(A) APPLICATION NUMBER:  
(B) FILING DATE:  
(C) CLASSIFICATION:
- (vii) PRIOR APPLICATION DATA:  
(A) APPLICATION NUMBER: 08/256,047  
(B) FILING DATE: NOVEMBER 17, 1992
- (viii) ATTORNEY/AGENT INFORMATION:  
(A) NAME: SIEGELL, BARBARA C.  
(B) REGISTRATION NUMBER: 30,684  
(C) REFERENCE/DOCKET NUMBER: BB-1043-C
- (ix) TELECOMMUNICATION INFORMATION:  
(A) TELEPHONE: (302) 992-4931  
(B) TELEFAX: (302) 773-0164  
(C) TELEX: 835420

002280 0254960

(i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 1464 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

(ix) **FEATURE:**  
(A) **NAME/KEY:** CDS  
(B) **LOCATION:** 130..1281

GGCACGAGCT	CGTGCCGAAT	TCGGCACGAG	AGGAGACAGA	GAGAGAGTTT	GAGGAGGAGC		60
TTCTTCGTAG	GGTTCATCGT	TATTAACGTT	AAATCTTCAT	CCCCCCTAC	GTCAGCCAGC		120
TCAAGAAAC	ATG GGT GCA GGT GGA AGA ATG CAA GTG TCT CCT CCC TCC						168
	Met Gly Ala Gly Gly Arg Met Gln Val Ser Pro Pro Ser						
	1		5		10		
AAA AAG TCT GAA ACC GAC AAC ATC AAG CGC GTA CCC TGC GAG ACA CCG							216
Lys Lys Ser Ser Glu Thr Asp Asn Ile Lys Arg Val Pro Cys Glu Thr Pro							
	15		20		25		
CCC TTC ACT GTC GGA GAA CTC AAG AAA GCA ATC CCA CCG CAC TGT TTC							264
Pro Phe Thr Val Gly Glu Leu Lys Lys Ala Ile Pro Pro His Cys Phe							
	30		35		40		45
AAA CGC TCG ATC CCT CGC TCT TTC TCC TAC CTC ATC TGG GAC ATC ATC							312
Lys Arg Ser Ile Pro Arg Ser Phe Ser Tyr Leu Ile Trp Asp Ile Ile							
	50				55		60
ATA GCC TCC TGC TTC TAC TAC GTC GCC ACC ACT TAC TTC CCT CTC CTC							360
Ile Ala Ser Cys Phe Tyr Tyr Val Ala Thr Thr Tyr Phe Pro Leu Leu							
	65				70		75
CCT CAC CCT CTC TCC TAC TTC GCC TGG CCT CTC TAC TGG GCC TGC CAG							408
Pro His Pro Leu Ser Tyr Phe Ala Trp Pro Leu Tyr Trp Ala Cys Gln							
	80				85		90
GGC TGC GTC CTA ACC GGC GTC TGG GTC ATA GCC CAC GAG TGC GGC CAC							456
Gly Cys Val Leu Thr Gly Val Trp Val Ile Ala His Glu Cys Gly His							
	95				100		105
CAC GCC TTC AGC GAC TAC CAG TGG CTG GAC GAC ACC GTC GGC CTC ATC							504
His Ala Phe Ser Asp Tyr Gln Trp Leu Asp Asp Thr Val Gly Leu Ile							
	110				115		120
TTC CAC TCC TTC CTC CTC GTC CCT TAC TTC TCC TGG AAG TAC AGT CAT							552
Phe His Ser Phe Leu Leu Val Pro Tyr Phe Ser Trp Lys Tyr Ser His							
	130						135
CGA CGC CAC CAT TCC AAC ACT GGC TCC CTC GAG AGA GAC GAA GTG TTT							600
Arg Arg His His Ser Asn Thr Gly Ser Leu Glu Arg Asp Glu Val Phe							
	145						150
GTC CCC AAG AAG AAG TCA GAC ATC AAG TGG TAC GGC AAG TAC CTC AAC							648
Val Pro Lys Lys Lys Ser Asp Ile Lys Trp Tyr Gly Lys Tyr Leu Asn							
	160				165		170

AAC CCT TTG TTA GCG ACC GTG ATG TTA ACG GTT CAG TTC ACT CTC GGC	696
Asn Pro Leu Gly Arg Thr Val Met Leu Thr Val Gln Phe Thr Leu Gly	
175 180 185	
TGG CCT TTG TAC TTA GCC TTC AAC GTC TCG GGG AGA CCT TAC GAC GGC	744
Trp Pro Leu Tyr Leu Ala Phe Asn Val Ser Gly Arg Pro Tyr Asp Gly	
190 195 200 205	
GGC TTC GCT TGC CAT TTC CAC CCC AAC GCT CCC ATC TAC AAC GAC CGT	792
Gly Phe Ala Cys His Phe His Pro Asn Ala Pro Ile Tyr Asn Asp Arg	
210 215 220	
GAG CGT CTC CAG ATA TAC ATC TCC GAC GCT GGC ATC CTC GCC GTC TGC	840
Glu Arg Leu Gln Ile Tyr Ile Ser Asp Ala Gly Ile Leu Ala Val Cys	
225 230 235	
TAC GGT CTC TAC CGC TAC GCT GCT GTC CAA GGA GTT GCC TCG ATG GTC	888
Tyr Gly Leu Tyr Arg Tyr Ala Val Gln Gly Val Ala Ser Met Val	
240 245 250	
TGC TTC TAC GGA GTT CCT CTT CTG ATT GTC AAC GGG TTC TTA GTT TTG	936
Cys Phe Tyr Gly Val Pro Leu Leu Ile Val Asn Gly Phe Leu Val Leu	
255 260 265	
ATC ACT TAC TTG CAG CAC ACG CAT CCT TCC CTG CCT CAC TAT GAC TCG	984
Ile Thr Tyr Leu Gln His Thr His Pro Ser Leu Pro His Tyr Asp Ser	
270 275 280 285	
TCT GAG TGG GAT TGG TTG AGG GGA GCT TTG GCC ACC GTT GAC AGA GAC	1032
Ser Glu Trp Asp Trp Leu Arg Gly Ala Leu Ala Thr Val Asp Arg Asp	
290 295 300	
TAC GGA ATC TTG AAC AAG GTC TTC CAC AAT ATC ACG GAC ACG CAC GTG	1080
Tyr Gly Ile Leu Asn Lys Val Phe His Asn Ile Thr Asp Thr His Val	
305 310 315	
GCG CAT CAC CTG TTC TCG ACC ATG CCG CAT TAT CAT GCG ATG GAA GCT	1128
Ala His His Leu Phe Ser Thr Met Pro His Tyr His Ala Met Glu Ala	
320 325 330	
ACG AAG GCG ATA AAG CCG ATA CTG GGA GAG TAT TAT CAG TTC GAT GGG	1176
Thr Lys Ala Ile Lys Pro Ile Leu Gly Glu Tyr Tyr Gln Phe Asp Gly	
335 340 345	
ACG CCG GTG GTT AAG GCG ATG TGG AGG GAG GCG AAG GAG TGT ATC TAT	1224
Thr Pro Val Val Lys Ala Met Trp Arg Glu Ala Lys Glu Cys Ile Tyr	
350 355 360 365	
GTG GAA CCG GAC AGG CAA GGT GAG AAG AAA GGT GTG TTC TGG TAC AAC	1272
Val Glu Pro Asp Arg Gln Gly Glu Lys Lys Gly Val Phe Trp Tyr Asn	
370 375 380	
AAT AAG TTA TGAAGCAAAG AAGAACTGA ACCTTTCTCT TCTATGATTG	1321
Asn Lys Leu	
TCTTTGTTTA AGAAGCTATG TTTCTGTTTC AATAATCTTA ATTATCCATT TTGTTGTGTT	1381
TTCTGACATT TTGGCTAAAA TTATGTGATG TTGGAAGTTA GTGTCTAAAA AAAAAAAAAA	1441
AAAAAAAAAAAA AAAAAAAAAA AAA	1464

(i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 384 amino acids  
(B) TYPE: amino acid  
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

Met	Gly	Ala	Gly	Gly	Arg	Met	Gln	Val	Ser	Pro	Pro	Ser	Lys	Lys	Ser
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Glu	Thr	Asp	Asn	Ile	Lys	Arg	Val	Pro	Cys	Glu	Thr	Pro	Pro	Phe	Thr
			20					25					30		
Val	Gly	Glu	Leu	Lys	Lys	Ala	Ile	Pro	Pro	His	Cys	Phe	Lys	Arg	Ser
			35				40					45			
Ile	Pro	Arg	Ser	Phe	Ser	Tyr	Leu	Ile	Trp	Asp	Ile	Ile	Ile	Ala	Ser
	50					55					60				
Cys	Phe	Tyr	Tyr	Val	Ala	Thr	Thr	Tyr	Phe	Pro	Leu	Leu	Pro	His	Pro
	65				70					75					80
Leu	Ser	Tyr	Phe	Ala	Trp	Pro	Leu	Tyr	Trp	Ala	Cys	Gln	Gly	Cys	Val
				85					90					95	
Leu	Thr	Gly	Val	Trp	Val	Ile	Ala	His	Glu	Cys	Gly	His	His	Ala	Phe
			100					105					110		
Ser	Asp	Tyr	Gln	Trp	Leu	Asp	Asp	Thr	Val	Gly	Leu	Ile	Phe	His	Ser
		115					120					125			
Phe	Leu	Leu	Val	Pro	Tyr	Phe	Ser	Trp	Lys	Tyr	Ser	His	Arg	Arg	His
	130					135					140				
His	Ser	Asn	Thr	Gly	Ser	Leu	Glu	Arg	Asp	Glu	Val	Phe	Val	Pro	Lys
145					150					155					160
Lys	Lys	Ser	Asp	Ile	Lys	Trp	Tyr	Gly	Lys	Tyr	Leu	Asn	Asn	Pro	Leu
				165					170					175	
Gly	Arg	Thr	Val	Met	Leu	Thr	Val	Gln	Phe	Thr	Leu	Gly	Trp	Pro	Leu
			180					185					190		
Tyr	Leu	Ala	Phe	Asn	Val	Ser	Gly	Arg	Pro	Tyr	Asp	Gly	Gly	Phe	Ala
		195					200					205			
Cys	His	Phe	His	Pro	Asn	Ala	Pro	Ile	Tyr	Asn	Asp	Arg	Glu	Arg	Leu
	210					215					220				
Gln	Ile	Tyr	Ile	Ser	Asp	Ala	Gly	Ile	Leu	Ala	Val	Cys	Tyr	Gly	Leu
225					230					235					240
Tyr	Arg	Tyr	Ala	Ala	Val	Gln	Gly	Val	Ala	Ser	Met	Val	Cys	Phe	Tyr
				245					250					255	
Gly	Val	Pro	Leu	Leu	Ile	Val	Asn	Gly	Phe	Leu	Val	Leu	Ile	Thr	Tyr
			260					265					270		
Leu	Gln	His	Thr	His	Pro	Ser	Leu	Pro	His	Tyr	Asp	Ser	Ser	Glu	Trp
		275					280					285			

Asp Trp Leu Arg Gly Ala Leu Ala Thr Val Asp Arg Asp Tyr Gly Ile  
 290 295 300  
 Leu Asn Lys Val Phe His Asn Ile Thr Asp Thr His Val Ala His His  
 305 310 315 320  
 Leu Phe Ser Thr Met Pro His Tyr His Ala Met Glu Ala Thr Lys Ala  
 325 330 335  
 Ile Lys Pro Ile Leu Gly Glu Tyr Tyr Gln Phe Asp Gly Thr Pro Val  
 340 345 350  
 Val Lys Ala Met Trp Arg Glu Ala Lys Glu Cys Ile Tyr Val Glu Pro  
 355 360 365  
 Asp Arg Gln Gly Glu Lys Lys Gly Val Phe Trp Tyr Asn Asn Lys Leu  
 370 375 380

## (2) INFORMATION FOR SEQ ID NO:3:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 1155 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: DNA
- (iii) HYPOTHETICAL: NO
- (iv) ANTI-SENSE: NO
- (vi) ORIGINAL SOURCE:
  - (A) ORGANISM: Brassica napus
- (vii) IMMEDIATE SOURCE:
  - (B) CLONE: IMC129
- (ix) FEATURE:
  - (D) OTHER INFORMATION: G to A transversion  
mutation at nucleotide 316  
of the D form
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

ATG GGT GCA GGT GGA AGA ATG CAA GTG TCT CCT CCC TCC AAA AAG TCT	48
Met Gly Ala Gly Gly Arg Met Gln Val Ser Pro Pro Ser Lys Lys Ser	
1 5 10 15	
GAA ACC GAC AAC ATC AAG CGC GTA CCC TGC GAG ACA CCG CCC TTC ACT	96
Glu Thr Asp Asn Ile Lys Arg Val Pro Cys Glu Thr Pro Pro Phe Thr	
20 25 30	
GTC GGA GAA CTC AAG AAA GCA ATC CCA CCG CAC TGT TTC AAA CGC TCG	144
Val Gly Glu Leu Lys Lys Ala Ile Pro Pro His Cys Phe Lys Arg Ser	
35 40 45	
ATC CCT CGC TCT TTC TCC TAC CTC ATC TGG GAC ATC ATC ATA GCC TCC	192
Ile Pro Arg Ser Phe Ser Tyr Leu Ile Trp Asp Ile Ile Ile Ala Ser	
50 55 60	

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TGC TTC TAC TAC GTC GCC ACC ACT TAC TTC CCT CTC CTC CCT CAC CCT	240
Cys Phe Tyr Tyr Val Ala Thr Thr Tyr Phe Pro Leu Leu Pro His Pro	
65 70 75 80	
CTC TCC TAC TTC GCC TGG CCT CTC TAC TGG GCC TGC CAG GGC TGC GTC	288
Leu Ser Tyr Phe Ala Trp Pro Leu Tyr Trp Ala Cys Gln Gly Cys Val	
85 90 95	
CTA ACC GGC GTC TGG GTC ATA GCC CAC AAG TGC GGC CAC CAC GCC TTC	336
Leu Thr Gly Val Trp Val Ile Ala His Lys Cys Gly His His Ala Phe	
100 105 110	
AGC GAC TAC CAG TGG CTG GAC GAC ACC GTC GGC CTC ATC TTC CAC TCC	384
Ser Asp Tyr Gln Trp Leu Asp Asp Thr Val Gly Leu Ile Phe His Ser	
115 120 125	
TTC CTC CTC GTC CCT TAC TTC TCC TGG AAG TAC AGT CAT CGA CGC CAC	432
Phe Leu Leu Val Pro Tyr Phe Ser Trp Lys Tyr Ser His Arg Arg His	
130 135 140	
CAT TCC AAC ACT GGC TCC CTC GAG AGA GAC GAA GTG TTT GTC CCC AAG	480
His Ser Asn Thr Gly Ser Leu Glu Arg Asp Glu Val Phe Val Pro Lys	
145 150 155 160	
AAG AAG TCA GAC ATC AAG TGG TAC GGC AAG TAC CTC AAC AAC CCT TTG	528
Lys Lys Ser Asp Ile Lys Trp Tyr Gly Lys Tyr Leu Asn Asn Pro Leu	
165 170 175	
GGA CGC ACC GTG ATG TTA ACG GTT CAG TTC ACT CTC GGC TGG CCT TTG	576
Gly Arg Thr Val Met Leu Thr Val Gln Phe Thr Leu Gly Trp Pro Leu	
180 185 190	
TAC TTA GCC TTC AAC GTC TCG GGG AGA CCT TAC GAC GGC GGC TTC GCT	624
Tyr Leu Ala Phe Asn Val Ser Gly Arg Pro Tyr Asp Gly Gly Phe Ala	
195 200 205	
TGC CAT TTC CAC CCC AAC GCT CCC ATC TAC AAC GAC CGC GAG CGT CTC	672
Cys His Phe His Pro Asn Ala Pro Ile Tyr Asn Asp Arg Glu Arg Leu	
210 215 220	
CAG ATA TAC ATC TCC GAC GCT GGC ATC CTC GCC GTC TGC TAC GGT CTC	720
Gln Ile Tyr Ile Ser Asp Ala Gly Ile Leu Ala Val Cys Tyr Gly Leu	
225 230 235 240	
TAC CGC TAC GCT GCT GTC CAA GGA GTT GCC TCG ATG GTC TGC TTC TAC	768
Tyr Arg Tyr Ala Ala Val Gln Gly Val Ala Ser Met Val Cys Phe Tyr	
245 250 255	
GGA GTT CCG CTT CTG ATT GTC AAT GGG TTC TTA GTT TTG ATC ACT TAC	816
Gly Val Pro Leu Leu Ile Val Asn Gly Phe Leu Val Leu Ile Thr Tyr	
260 265 270	
TTG CAG CAC ACG CAT CCT TCC CTG CCT CAC TAT GAC TCG TCT GAG TGG	864
Leu Gln His Thr His Pro Ser Leu Pro His Tyr Asp Ser Ser Glu Trp	
275 280 285	
GAT TGG TTG AGG GGA GCT TTG GCC ACC GTT GAC AGA GAC TAC GGA ATC	912
Asp Trp Leu Arg Gly Ala Leu Ala Thr Val Asp Arg Asp Tyr Gly Ile	
290 295 300	
TTG AAC AAG GTC TTC CAC AAT ATC ACG GAC ACG CAC GTG GCG CAT CAC	960
Leu Asn Lys Val Phe His Asn Ile Thr Asp Thr His Val Ala His His	
305 310 315 320	

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CTG TTC TCG ACC ATG CCG CAT TAT CAT GCG ATG GAA GCT ACG AAG GCG 1008  
 Leu Phe Ser Thr Met Pro His Tyr His Ala Met Glu Ala Thr Lys Ala  
                   325                                  330                                  335

ATA AAG CCG ATA CTG GGA GAG TAT TAT CAG TTC GAT GGG ACG CCG GTG 1056  
 Ile Lys Pro Ile Leu Gly Glu Tyr Tyr Gln Phe Asp Gly Thr Pro Val  
                   340                                  345                                  350

GTT AAG GCG ATG TGG AGG GAG GCG AAG GAG TGT ATC TAT GTG GAA CCG 1104  
 Val Lys Ala Met Trp Arg Glu Ala Lys Glu Cys Ile Tyr Val Glu Pro  
                   355                                  360                                  365

GAC AGG CAA GGT GAG AAG AAA GGT GTG TTC TGG TAC AAC AAT AAG TTA T 1153  
 Asp Arg Gln Gly Glu Lys Lys Gly Val Phe Trp Tyr Asn Asn Lys Leu  
                   370                                  375                                  380

GA 1155

## (2) INFORMATION FOR SEQ ID NO:4:

- (i) SEQUENCE CHARACTERISTICS:  
 (A) LENGTH: 384 amino acids  
 (B) TYPE: amino acid  
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

Met Gly Ala Gly Gly Arg Met Gln Val Ser Pro Pro Ser Lys Lys Ser  
   1                  5                                  10                                  15

Glu Thr Asp Asn Ile Lys Arg Val Pro Cys Glu Thr Pro Pro Phe Thr  
                   20                                  25                                  30

Val Gly Glu Leu Lys Lys Ala Ile Pro Pro His Cys Phe Lys Arg Ser  
                   35                                  40                                  45

Ile Pro Arg Ser Phe Ser Tyr Leu Ile Trp Asp Ile Ile Ile Ala Ser  
                   50                                  55                                  60

Cys Phe Tyr Tyr Val Ala Thr Thr Tyr Phe Pro Leu Leu Pro His Pro  
                   65                                  70                                  75                                  80

Leu Ser Tyr Phe Ala Trp Pro Leu Tyr Trp Ala Cys Gln Gly Cys Val  
                   85                                  90                                  95

Leu Thr Gly Val Trp Val Ile Ala His Lys Cys Gly His His Ala Phe  
                   100                                  105                                  110

Ser Asp Tyr Gln Trp Leu Asp Asp Thr Val Gly Leu Ile Phe His Ser  
                   115                                  120                                  125

Phe Leu Leu Val Pro Tyr Phe Ser Trp Lys Tyr Ser His Arg Arg His  
                   130                                  135                                  140

His Ser Asn Thr Gly Ser Leu Glu Arg Asp Glu Val Phe Val Pro Lys  
                   145                                  150                                  155                                  160

Lys Lys Ser Asp Ile Lys Trp Tyr Gly Lys Tyr Leu Asn Asn Pro Leu  
                   165                                  170                                  175

Gly Arg Thr Val Met Leu Thr Val Gln Phe Thr Leu Gly Trp Pro Leu  
                   180                                  185                                  190

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Tyr Leu Ala Phe Asn Val Ser Gly Arg Pro Tyr Asp Gly Gly Phe Ala  
 195 200 205  
 Cys His Phe His Pro Asn Ala Pro Ile Tyr Asn Asp Arg Glu Arg Leu  
 210 215 220  
 Gln Ile Tyr Ile Ser Asp Ala Gly Ile Leu Ala Val Cys Tyr Gly Leu  
 225 230 235 240  
 Tyr Arg Tyr Ala Ala Val Gln Gly Val Ala Ser Met Val Cys Phe Tyr  
 245 250 255  
 Gly Val Pro Leu Leu Ile Val Asn Gly Phe Leu Val Leu Ile Thr Tyr  
 260 265 270  
 Leu Gln His Thr His Pro Ser Leu Pro His Tyr Asp Ser Ser Glu Trp  
 275 280 285  
 Asp Trp Leu Arg Gly Ala Leu Ala Thr Val Asp Arg Asp Tyr Gly Ile  
 290 295 300  
 Leu Asn Lys Val Phe His Asn Ile Thr Asp Thr His Val Ala His His  
 305 310 315 320  
 Leu Phe Ser Thr Met Pro His Tyr His Ala Met Glu Ala Thr Lys Ala  
 325 330 335  
 Ile Lys Pro Ile Leu Gly Glu Tyr Tyr Gln Phe Asp Gly Thr Pro Val  
 340 345 350  
 Val Lys Ala Met Trp Arg Glu Ala Lys Glu Cys Ile Tyr Val Glu Pro  
 355 360 365  
 Asp Arg Gln Gly Glu Lys Lys Gly Val Phe Trp Tyr Asn Asn Lys Leu  
 370 375 380

## (2) INFORMATION FOR SEQ ID NO:5:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 1155 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: DNA
- (iii) HYPOTHETICAL: NO
- (iv) ANTI-SENSE: NO
- (vi) ORIGINAL SOURCE:
  - (A) ORGANISM: Brassica napus
- (ix) FEATURE:
  - (D) OTHER INFORMATION: Wild type F form.
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

ATG GGT GCA GGT GGA AGA ATG CAA GTG TCT CCT CCC TCC AAG AAG TCT  
 Met Gly Ala Gly Gly Arg Met Gln Val Ser Pro Pro Ser Lys Lys Ser  
 1 5 10 15

GAA ACC GAC ACC ATC AAG CGC GTA CCC TGC GAG ACA CCG CCC TTC ACT	96
Glu Thr Asp Thr Ile Lys Arg Val Pro Cys Glu Thr Pro Pro Phe Thr	
20 25 30	
GTC GGA GAA CTC AAG AAA GCA ATC CCA CCG CAC TGT TTC AAA CGC TCG	144
Val Gly Glu Leu Lys Lys Ala Ile Pro Pro His Cys Phe Lys Arg Ser	
35 40 45	
ATC CCT CGC TCT TTC TCC TAC CTC ATC TGG GAC ATC ATC ATA GCC TCC	192
Ile Pro Arg Ser Phe Ser Tyr Leu Ile Trp Asp Ile Ile Ile Ala Ser	
50 55 60	
TGC TTC TAC TAC GTC GCC ACC ACT TAC TTC CCT CTC CTC CCT CAC CCT	240
Cys Phe Tyr Tyr Val Ala Thr Thr Tyr Phe Pro Leu Leu Pro His Pro	
65 70 75 80	
CTC TCC TAC TTC GCC TGG CCT CTC TAC TGG GCC TGC CAA GGG TGC GTC	288
Leu Ser Tyr Phe Ala Trp Pro Leu Tyr Trp Ala Cys Gln Gly Cys Val	
85 90 95	
CTA ACC GGC GTC TGG GTC ATA GCC CAC GAG TGC GGC CAC CAC GCC TTC	336
Leu Thr Gly Val Trp Val Ile Ala His Glu Cys Gly His His Ala Phe	
100 105 110	
AGC GAC TAC CAG TGG CTT GAC GAC ACC GTC GGT CTC ATC TTC CAC TCC	384
Ser Asp Tyr Gln Trp Leu Asp Thr Val Gly Leu Ile Phe His Ser	
115 120 125	
TTC CTC CTC GTC CCT TAC TTC TCC TGG AAG TAC AGT CAT CGC AGC CAC	432
Phe Leu Leu Val Pro Tyr Phe Ser Trp Lys Tyr Ser His Arg Ser His	
130 135 140	
CAT TCC AAC ACT GGC TCC CTC GAG AGA GAC GAA GTG TTT GTC CCC AAG	480
His Ser Asn Thr Gly Ser Leu Glu Arg Asp Glu Val Phe Val Pro Lys	
145 150 155 160	
AAG AAG TCA GAC ATC AAG TGG TAC GGC AAG TAC CTC AAC AAC CCT TTG	528
Lys Lys Ser Asp Ile Lys Trp Tyr Gly Lys Tyr Leu Asn Asn Pro Leu	
165 170 175	
GGA CGC ACC GTG ATG TTA ACG GTT CAG TTC ACT CTC GGC TGG CCG TTG	576
Gly Arg Thr Val Met Leu Thr Val Gln Phe Thr Leu Gly Trp Pro Leu	
180 185 190	
TAC TTA GCC TTC AAC GTC TCG GGA AGA CCT TAC GAC GGC GGC TTC CGT	624
Tyr Leu Ala Phe Asn Val Ser Gly Arg Pro Tyr Asp Gly Gly Phe Arg	
195 200 205	
TGC CAT TTC CAC CCC AAC GCT CCC ATC TAC AAC GAC CGC GAG CGT CTC	672
Cys His Phe His Pro Asn Ala Pro Ile Tyr Asn Asp Arg Glu Arg Leu	
210 215 220	
CAG ATA TAC ATC TCC GAC GCT GGC ATC CTC GCC GTC TGC TAC GGT CTC	720
Gln Ile Tyr Ile Ser Asp Ala Gly Ile Leu Ala Val Cys Tyr Gly Leu	
225 230 235 240	
TTC CGT TAC GCC GCC GGC CAG GGA GTG GCC TCG ATG GTC TGC TTC TAC	768
Phe Arg Tyr Ala Ala Gly Gln Gly Val Ala Ser Met Val Cys Phe Tyr	
245 250 255	
GGA GTC CCG CTT CTG ATT GTC AAT GGT TTC CTC GTG TTG ATC ACT TAC	816
Gly Val Pro Leu Leu Ile Val Asn Gly Phe Leu Val Leu Ile Thr Tyr	
260 265 270	

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TTG CAG CAC ACG CAT CCT TCC CTG CCT CAC TAC GAT TCG TCC GAG TGG 864  
 Leu Gln His Thr His Pro Ser Leu Pro His Tyr Asp Ser Ser Glu Trp  
 275 280 285  
 GAT TGG TTC AGG GGA GCT TTG GCT ACC GTT GAC AGA GAC TAC GGA ATC 912  
 Asp Trp Phe Arg Gly Ala Leu Ala Thr Val Asp Arg Asp Tyr Gly Ile  
 290 295 300  
 TTG AAC AAG GTC TTC CAC AAT ATT ACC GAC ACG CAC GTG GCC CAT CAT 960  
 Leu Asn Lys Val Phe His Asn Ile Thr Asp Thr His Val Ala His His  
 305 310 315 320  
 CCG TTC TCC ACG ATG CCG CAT TAT CAC GCG ATG GAA GCT ACC AAG GCG 1008  
 Pro Phe Ser Thr Met Pro His Tyr His Ala Met Glu Ala Thr Lys Ala  
 325 330 335  
 ATA AAG CCG ATA CTG GGA GAG TAT TAT CAG TTC GAT GGG ACG CCG GTG 1056  
 Ile Lys Pro Ile Leu Gly Glu Tyr Tyr Gln Phe Asp Gly Thr Pro Val  
 340 345 350  
 GTT AAG GCG ATG TGG AGG GAG GCG AAG GAG TGT ATC TAT GTG GAA CCG 1104  
 Val Lys Ala Met Trp Arg Glu Ala Lys Glu Cys Ile Tyr Val Glu Pro  
 355 360 365  
 GAC AGG CAA GGT GAG AAG AAA GGT GTG TTC TGG TAC AAC AAT AAG TTA T 1153  
 Asp Arg Gln Gly Glu Lys Lys Gly Val Phe Trp Tyr Asn Asn Lys Leu  
 370 375 380  
 GA 1155

## (2) INFORMATION FOR SEQ ID NO:6:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 384 amino acids  
 (B) TYPE: amino acid  
 (D) TOPOLOGY: linear

## (ii) MOLECULE TYPE: protein

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

Met Gly Ala Gly Gly Arg Met Gln Val Ser Pro Pro Ser Lys Lys Ser  
 1 5 10 15  
 Glu Thr Asp Thr Ile Lys Arg Val Pro Cys Glu Thr Pro Pro Phe Thr  
 20 25 30  
 Val Gly Glu Leu Lys Lys Ala Ile Pro Pro His Cys Phe Lys Arg Ser  
 35 40 45  
 Ile Pro Arg Ser Phe Ser Tyr Leu Ile Trp Asp Ile Ile Ile Ala Ser  
 50 55 60  
 Cys Phe Tyr Tyr Val Ala Thr Thr Tyr Phe Pro Leu Leu Pro His Pro  
 65 70 75 80  
 Leu Ser Tyr Phe Ala Trp Pro Leu Tyr Trp Ala Cys Gln Gly Cys Val  
 85 90 95  
 Leu Thr Gly Val Trp Val Ile Ala His Glu Cys Gly His His Ala Phe  
 100 105 110  
 Ser Asp Tyr Gln Trp Leu Asp Asp Thr Val Gly Leu Ile Phe His Ser  
 115 120 125

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Phe Leu Leu Val Pro Tyr Phe Ser Trp Lys Tyr Ser His Arg Ser His  
 130 135 140  
 His Ser Asn Thr Gly Ser Leu Glu Arg Asp Glu Val Phe Val Pro Lys  
 145 150 155 160  
 Lys Lys Ser Asp Ile Lys Trp Tyr Gly Lys Tyr Leu Asn Asn Pro Leu  
 165 170 175  
 Gly Arg Thr Val Met Leu Thr Val Gln Phe Thr Leu Gly Trp Pro Leu  
 180 185 190  
 Tyr Leu Ala Phe Asn Val Ser Gly Arg Pro Tyr Asp Gly Gly Phe Arg  
 195 200 205  
 Cys His Phe His Pro Asn Ala Pro Ile Tyr Asn Asp Arg Glu Arg Leu  
 210 215 220  
 Gln Ile Tyr Ile Ser Asp Ala Gly Ile Leu Ala Val Cys Tyr Gly Leu  
 225 230 235 240  
 Phe Arg Tyr Ala Ala Gly Gln Gly Val Ala Ser Met Val Cys Phe Tyr  
 245 250 255  
 Gly Val Pro Leu Leu Ile Val Asn Gly Phe Leu Val Leu Ile Thr Tyr  
 260 265 270  
 Leu Gln His Thr His Pro Ser Leu Pro His Tyr Asp Ser Ser Glu Trp  
 275 280 285  
 Asp Trp Phe Arg Gly Ala Leu Ala Thr Val Asp Arg Asp Tyr Gly Ile  
 290 295 300  
 Leu Asn Lys Val Phe His Asn Ile Thr Asp Thr His Val Ala His His  
 305 310 315 320  
 Pro Phe Ser Thr Met Pro His Tyr His Ala Met Glu Ala Thr Lys Ala  
 325 330 335  
 Ile Lys Pro Ile Leu Gly Glu Tyr Tyr Gln Phe Asp Gly Thr Pro Val  
 340 345 350  
 Val Lys Ala Met Trp Arg Glu Ala Lys Glu Cys Ile Tyr Val Glu Pro  
 355 360 365  
 Asp Arg Gln Gly Glu Lys Lys Gly Val Phe Trp Tyr Asn Asn Lys Leu  
 370 375 380

(2) INFORMATION FOR SEQ ID NO:7:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 1155 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: DNA
- (iii) HYPOTHETICAL: NO
- (iv) ANTI-SENSE: NO

## (vi) ORIGINAL SOURCE:

(A) ORGANISM: Brassica napus

## (vii) IMMEDIATE SOURCE:

(B) CLONE: IMC Q508

## (ix) FEATURE:

(D) OTHER INFORMATION: T to A transversion  
mutation at nucleotide 515  
of the F form

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

ATG GGT GCA GGT GGA AGA ATG CAA GTG TCT CCT CCC TCC AAG AAG TCT	48
Met Gly Ala Gly Gly Arg Met Gln Val Ser Pro Pro Ser Lys Lys Ser	
1 5 10 15	
GAA ACC GAC ACC ATC AAG CGC GTA CCC TGC GAG ACA CCG CCC TTC ACT	96
Glu Thr Asp Thr Ile Lys Arg Val Pro Cys Glu Thr Pro Phe Thr	
20 25 30	
GTC GGA GAA CTC AAG AAA GCA ATC CCA CCG CAC TGT TTC AAA CGC TCG	144
Val Gly Glu Leu Lys Lys Ala Ile Pro Pro His Cys Phe Lys Arg Ser	
35 40 45	
ATC CCT CGC TCT TTC TCC TAC CTC ATC TGG GAC ATC ATC ATA GCC TCC	192
Ile Pro Arg Ser Phe Ser Tyr Leu Ile Trp Asp Ile Ile Ile Ala Ser	
50 55 60	
TGC TTC TAC TAC GTC GCC ACC ACT TAC TTC CCT CTC CTC CCT CAC CCT	240
Cys Phe Tyr Tyr Val Ala Thr Thr Tyr Phe Pro Leu Leu Pro His Pro	
65 70 75 80	
CTC TCC TAC TTC GCC TGG CCT CTC TAC TGG GCC TGC CAA GGG TGC GTC	288
Leu Ser Tyr Phe Ala Trp Pro Leu Tyr Trp Ala Cys Gln Gly Cys Val	
85 90 95	
CTA ACC GGC GTC TGG GTC ATA GCC CAC GAG TGC GGC CAC CAC GCC TTC	336
Leu Thr Gly Val Trp Val Ile Ala His Glu Cys Gly His His Ala Phe	
100 105 110	
AGC GAC TAC CAG TGG CTT GAC GAC ACC GTC GGT CTC ATC TTC CAC TCC	384
Ser Asp Tyr Gln Trp Leu Asp Asp Thr Val Gly Leu Ile Phe His Ser	
115 120 125	
TTC CTC CTC GTC CCT TAC TTC TCC TGG AAG TAC AGT CAT CGC AGC CAC	432
Phe Leu Leu Val Pro Tyr Phe Ser Trp Lys Tyr Ser His Arg Ser His	
130 135 140	
CAT TCC AAC ACT GGC TCC CTC GAG AGA GAC GAA GTG TTT GTC CCC AAG	480
His Ser Asn Thr Gly Ser Leu Glu Arg Asp Glu Val Phe Val Pro Lys	
145 150 155 160	
AAG AAG TCA GAC ATC AAG TGG TAC GGC AAG TAC CAC AAC AAC CCT TTG	528
Lys Lys Ser Asp Ile Lys Trp Tyr Gly Lys Tyr His Asn Asn Pro Leu	
165 170 175	
GGA CGC ACC GTG ATG TTA ACG GTT CAG TTC ACT CTC GGC TGG CCG TTG	576
Gly Arg Thr Val Met Leu Thr Val Gln Phe Thr Leu Gly Trp Pro Leu	
180 185 190	
TAC TTA GCC TTC AAC GTC TCG GGA AGA CCT TAC GAC GGC GGC TTC CGT	624
Tyr Leu Ala Phe Asn Val Ser Gly Arg Pro Tyr Asp Gly Gly Phe Arg	
195 200 205	

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TGC CAT TTC CAC CCC AAC GCT CCC ATC TAC AAC GAC CGC GAG CGT CTC 672  
 Cys His Phe His Pro Asn Ala Pro Ile Tyr Asn Asp Arg Glu Arg Leu  
 210 215 220  
 CAG ATA TAC ATC TCC GAC GCT GGC ATC CTC GCC GTC TGC TAC GGT CTC 720  
 Gln Ile Tyr Ile Ser Asp Ala Gly Ile Leu Ala Val Cys Tyr Gly Leu  
 225 230 235 240  
 TTC CGT TAC GCC GCC GGC CAG GGA GTG GCC TCG ATG GTC TGC TTC TAC 768  
 Phe Arg Tyr Ala Ala Gly Gln Gly Val Ala Ser Met Val Cys Phe Tyr  
 245 250 255  
 GGA GTC CCG CTT CTG ATT GTC AAT GGT TTC CTC GTG TTG ATC ACT TAC 816  
 Gly Val Pro Leu Leu Ile Val Asn Gly Phe Leu Val Leu Ile Thr Tyr  
 260 265 270  
 TTG CAG CAC ACG CAT CCT TCC CTG CCT CAC TAC GAT TCG TCC GAG TGG 864  
 Leu Gln His Thr His Pro Ser Leu Pro His Tyr Asp Ser Ser Glu Trp  
 275 280 285  
 GAT TGG TTC AGG GGA GCT TTG GCT ACC GTT GAC AGA GAC TAC GGA ATC 912  
 Asp Trp Phe Arg Gly Ala Leu Ala Thr Val Asp Arg Asp Tyr Gly Ile  
 290 295 300  
 TTG AAC AAG GTC TTC CAC AAT ATT ACC GAC ACG CAC GTG GCC CAT CAT 960  
 Leu Asn Lys Val Phe His Asn Ile Thr Asp Thr His Val Ala His His  
 305 310 315 320  
 CCG TTC TCC ACG ATG CCG CAT TAT CAC GCG ATG GAA GCT ACC AAG GCG 1008  
 Pro Phe Ser Thr Met Pro His Tyr His Ala Met Glu Ala Thr Lys Ala  
 325 330 335  
 ATA AAG CCG ATA CTG GGA GAG TAT TAT CAG TTC GAT GGG ACG CCG GTG 1056  
 Ile Lys Pro Ile Leu Gly Glu Tyr Tyr Gln Phe Asp Gly Thr Pro Val  
 340 345 350  
 GTT AAG GCG ATG TGG AGG GAG GCG AAG GAG TGT ATC TAT GTG GAA CCG 1104  
 Val Lys Ala Met Trp Arg Glu Ala Lys Glu Cys Ile Tyr Val Glu Pro  
 355 360 365  
 GAC AGG CAA GGT GAG AAG AAA GGT GTG TTC TGG TAC AAC AAT AAG TTA T 1153  
 Asp Arg Gln Gly Glu Lys Lys Gly Val Phe Trp Tyr Asn Asn Lys Leu  
 370 375 380  
 GA 1155

## (2) INFORMATION FOR SEQ ID NO:8:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 384 amino acids  
 (B) TYPE: amino acid  
 (D) TOPOLOGY: linear

## (ii) MOLECULE TYPE: protein

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

Met Gly Ala Gly Gly Arg Met Gln Val Ser Pro Pro Ser Lys Lys Ser  
 1 5 10 15  
 Glu Thr Asp Thr Ile Lys Arg Val Pro Cys Glu Thr Pro Pro Phe Thr  
 20 25 30

Val Gly Glu Leu Lys Lys Ala Ile Pro Pro His Cys Phe Lys Arg Ser  
 35 40 45  
 Ile Pro Arg Ser Phe Ser Tyr Leu Ile Trp Asp Ile Ile Ile Ala Ser  
 50 55 60  
 Cys Phe Tyr Tyr Val Ala Thr Thr Tyr Phe Pro Leu Leu Pro His Pro  
 65 70 75 80  
 Leu Ser Tyr Phe Ala Trp Pro Leu Tyr Trp Ala Cys Gln Gly Cys Val  
 85 90 95  
 Leu Thr Gly Val Trp Val Ile Ala His Glu Cys Gly His His Ala Phe  
 100 105 110  
 Ser Asp Tyr Gln Trp Leu Asp Asp Thr Val Gly Leu Ile Phe His Ser  
 115 120 125  
 Phe Leu Leu Val Pro Tyr Phe Ser Trp Lys Tyr Ser His Arg Ser His  
 130 135 140  
 His Ser Asn Thr Gly Ser Leu Glu Arg Asp Glu Val Phe Val Pro Lys  
 145 150 155 160  
 Lys Lys Ser Asp Ile Lys Trp Tyr Gly Lys Tyr His Asn Asn Pro Leu  
 165 170 175  
 Gly Arg Thr Val Met Leu Thr Val Gln Phe Thr Leu Gly Trp Pro Leu  
 180 185 190  
 Tyr Leu Ala Phe Asn Val Ser Gly Arg Pro Tyr Asp Gly Gly Phe Arg  
 195 200 205  
 Cys His Phe His Pro Asn Ala Pro Ile Tyr Asn Asp Arg Glu Arg Leu  
 210 215 220  
 Gln Ile Tyr Ile Ser Asp Ala Gly Ile Leu Ala Val Cys Tyr Gly Leu  
 225 230 235 240  
 Phe Arg Tyr Ala Ala Gly Gln Gly Val Ala Ser Met Val Cys Phe Tyr  
 245 250 255  
 Gly Val Pro Leu Leu Ile Val Asn Gly Phe Leu Val Leu Ile Thr Tyr  
 260 265 270  
 Leu Gln His Thr His Pro Ser Leu Pro His Tyr Asp Ser Ser Glu Trp  
 275 280 285  
 Asp Trp Phe Arg Gly Ala Leu Ala Thr Val Asp Arg Asp Tyr Gly Ile  
 290 295 300  
 Leu Asn Lys Val Phe His Asn Ile Thr Asp Thr His Val Ala His His  
 305 310 315 320  
 Pro Phe Ser Thr Met Pro His Tyr His Ala Met Glu Ala Thr Lys Ala  
 325 330 335  
 Ile Lys Pro Ile Leu Gly Glu Tyr Tyr Gln Phe Asp Gly Thr Pro Val  
 340 345 350  
 Val Lys Ala Met Trp Arg Glu Ala Lys Glu Cys Ile Tyr Val Glu Pro  
 355 360 365

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Asp Arg Gln Gly Glu Lys Lys Gly Val Phe Trp Tyr Asn Asn Lys Leu  
 370 375 380

(2) INFORMATION FOR SEQ ID NO:9:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 23 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: DNA (genomic)
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

CATGGGTGCA GGTGGAAGAA TGC

23

(2) INFORMATION FOR SEQ ID NO:10:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 21 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: DNA (genomic)
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

GTTTCTTCTT TGCTTCATAA C

21

(2) INFORMATION FOR SEQ ID NO:11:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 23 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: DNA (genomic)
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:

CATGGGTGCA GGTGGAAGAA TGC

23

(2) INFORMATION FOR SEQ ID NO:12:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 21 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: DNA (genomic)
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:12:

TCTTTCACCA TCATCATATC C

21

(2) INFORMATION FOR SEQ ID NO:13:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 19 base pairs
  - (B) TYPE: nucleic acid

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- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:13:

GTCTGGGTCA TAGCCCACG

19

(2) INFORMATION FOR SEQ ID NO:14:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 19 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:14:

GTCTGGGTCA TAGCCCACA

19

(2) INFORMATION FOR SEQ ID NO:15:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 17 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:15:

CTGGGTCATA GCCCATG

17

(2) INFORMATION FOR SEQ ID NO:16:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 17 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:16:

CTGGGTCATA GCCCACA

17

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2. The plant of Claim 1, wherein said construct comprises a full-length coding sequence of said mutant gene.

4. The plant of Claim 3, wherein said altered fatty acid composition comprises from about 1.0% to about 10.0% linoleic acid, based on total fatty acid composition.

6. The plant of Claim 1, wherein said mutant  
20 desaturase gene encodes a microsomal gene product.

8. The plant of Claim 7, wherein said mutant  
25 desaturase gene comprises the sequence  
His-Lys-Cys-Gly-His.

10. A plant containing a recombinant nucleic acid construct, said construct comprising at least one seed-specific regulatory sequence operably linked in sense orientation to a mutant delta-15 fatty acid desaturase gene and wherein said construct confers altered fatty acid composition in seeds of said plant.

11. The plant of Claim 10 wherein the plant is a Brassica canola plant.

12. The plant of Claim 10, wherein said construct comprises a full-length coding sequence of said mutant gene.

13. The plant of Claim 11, wherein said altered fatty acid composition comprises from about 1% to 10%  $\alpha$ -linolenic acid, based on total fatty acid composition.

14. The plant of Claim 10, wherein said mutant desaturase gene encodes a microsomal gene product.

15. The plant of Claim 10, wherein said mutant desaturase gene comprises a non-conservative amino acid substitution.

16. The plant of Claim 15, wherein said mutant desaturase gene comprises the sequence His-Lys-Cys-Gly-His.

17. The plant of Claim 10, wherein said mutant desaturase gene is from a Brassica napus plant.

18. A plant containing one or more recombinant nucleic acid constructs, said one or more constructs comprising:

a) at least one seed-specific regulatory sequence operably linked in sense orientation to a mutant delta-12 fatty acid desaturase gene; and

b) at least one seed-specific regulatory sequence operably linked in sense orientation to a mutant delta-15 fatty acid desaturase gene,

said mutant delta-12 and mutant delta-15 desaturase genes conferring altered fatty acid composition in seeds of said plant.

19. The plant of Claim 18 wherein the plant is a Brassica canola plant.

20. The plant of Claim 18, wherein said construct comprises a full-length coding sequence of said mutant delta-12 fatty acid desaturase gene.

21. The plant of Claim 18, wherein said construct comprises a full-length coding sequence of said mutant delta-15 fatty acid desaturase gene.

22. The plant of Claim 19, wherein said altered fatty acid composition comprises from about 1.0% to about 10.0% linoleic acid and from about 1.0% to about 10.0%  $\alpha$ -linolenic acid, based on total fatty acid composition.

5 23. A method for altering fatty acid composition in plant seeds, comprising the steps of:

- 10 a) introducing a recombinant nucleic acid construct into a plant, said construct comprising at least one seed-specific regulatory sequence operably linked in sense orientation to a mutant delta-12 fatty acid desaturase gene;
- 15 b) obtaining progeny from said plant, said progeny producing seeds having said altered fatty acid composition; and
- c) producing seeds having said altered fatty acid composition.

20 24. The method of Claim 23, wherein said construct comprises a full-length coding sequence of said mutant gene.

25 25. The method of Claim 23, wherein said altered fatty acid composition comprises a decreased level of linoleic acid.

26. A method for altering fatty acid composition in seeds, comprising the steps of:

- 30 a) introducing a recombinant nucleic acid construct into a plant, said construct comprising at least one seed-specific regulatory sequence operably linked in sense orientation to a mutant delta-15 fatty acid desaturase gene;
- b) obtaining progeny from said plant, said progeny producing seeds having said altered fatty acid composition; and
- 35 c) producing said seeds having said altered fatty acid composition.

27. The method of Claim 26, wherein said construct comprises a full-length coding sequence of said mutant gene.

28. The method of Claim 26, wherein said altered fatty acid composition comprises decreased levels of  $\alpha$ -linolenic acid.

29. A recombinant nucleic acid construct effective for altering fatty acid composition in seeds, said construct comprising at least one seed-specific regulatory sequence operably linked in sense orientation to a mutant delta-12 fatty acid desaturase gene.

30. A recombinant nucleic acid construct effective for altering fatty acid composition in seeds, said construct comprising at least one seed-specific regulatory sequence operably linked in sense orientation to a mutant delta-15 fatty acid desaturase gene.

31. A vegetable oil extracted from seeds produced by the plant of Claim 1.

32. A vegetable oil extracted from seeds produced by the plant of Claim 10.

33. A vegetable oil extracted from seeds produced by the plant of Claim 17.

34. A vegetable oil produced by the method of Claim 21.

35. A mutant delta-12 fatty acid desaturase comprising the amino acid sequence of SEQ ID selected from the group consisting of SEQ ID NO:4 and SEQ ID NO:8 or any mutant substantially similar thereto.

36. A nucleic acid fragment encoding the mutant delta-12 fatty acid desaturase of Claim 35.

002280-6254960

**TITLE**

GENES FOR MICROSOMAL DELTA-12 FATTY ACID  
DESATURASES AND RELATED ENZYMES FROM PLANTS  
ABSTRACT OF THE DISCLOSURE

5 The preparation and use of nucleic acid fragments encoding fatty acid desaturase enzymes are described. The invention permits alteration of plant lipid composition. Chimeric genes incorporating such nucleic acid fragments with suitable regulatory sequences may be used to create  
10 transgenic plants with altered levels of unsaturated fatty acids.

15

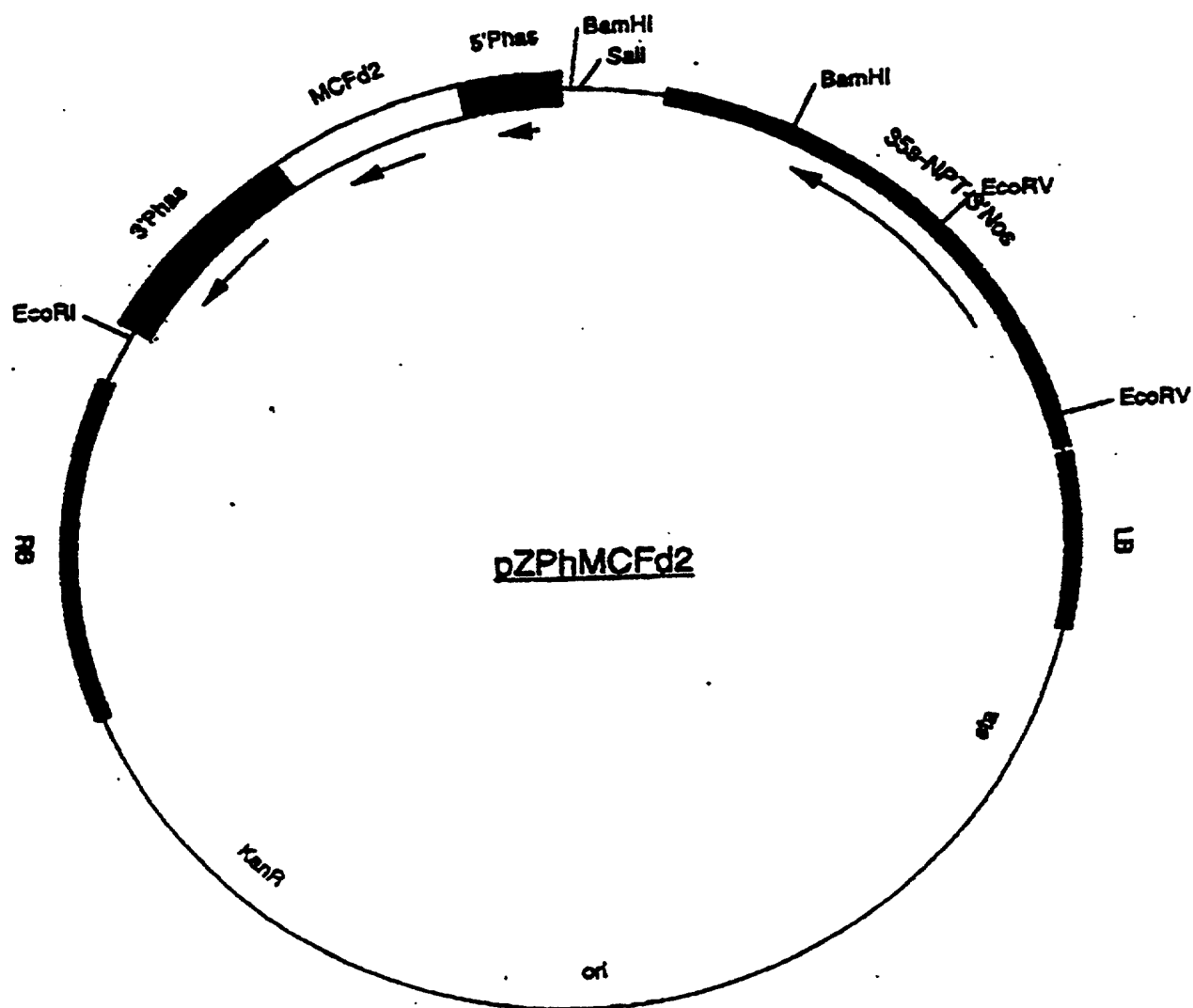
20

25

30

35 WRM/BCS: rnm/bjm

00643579-082200



Sense Can Fd2 (129 allele)  
Phas promoter & 3'end  
Into pZS212 EcoRI, SalI

Figure 1

phaseolin - 1Mc129 Sense





Figure 3

Distribution of T2 Populations with Transformed Fad2 D-Allele Point-Mutation  
and Fad2 D-Allele Genes, Classified by 18:2 Content

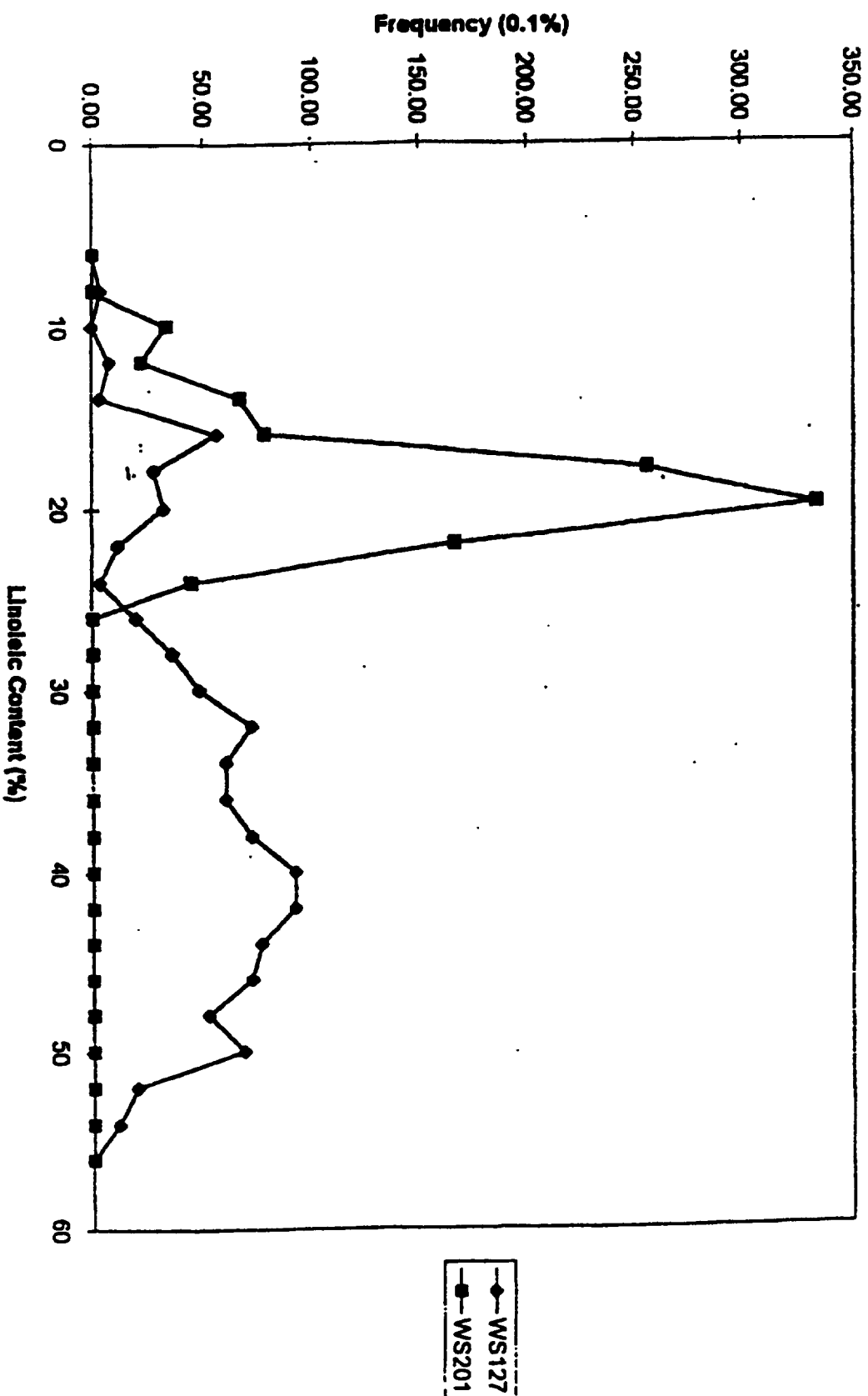
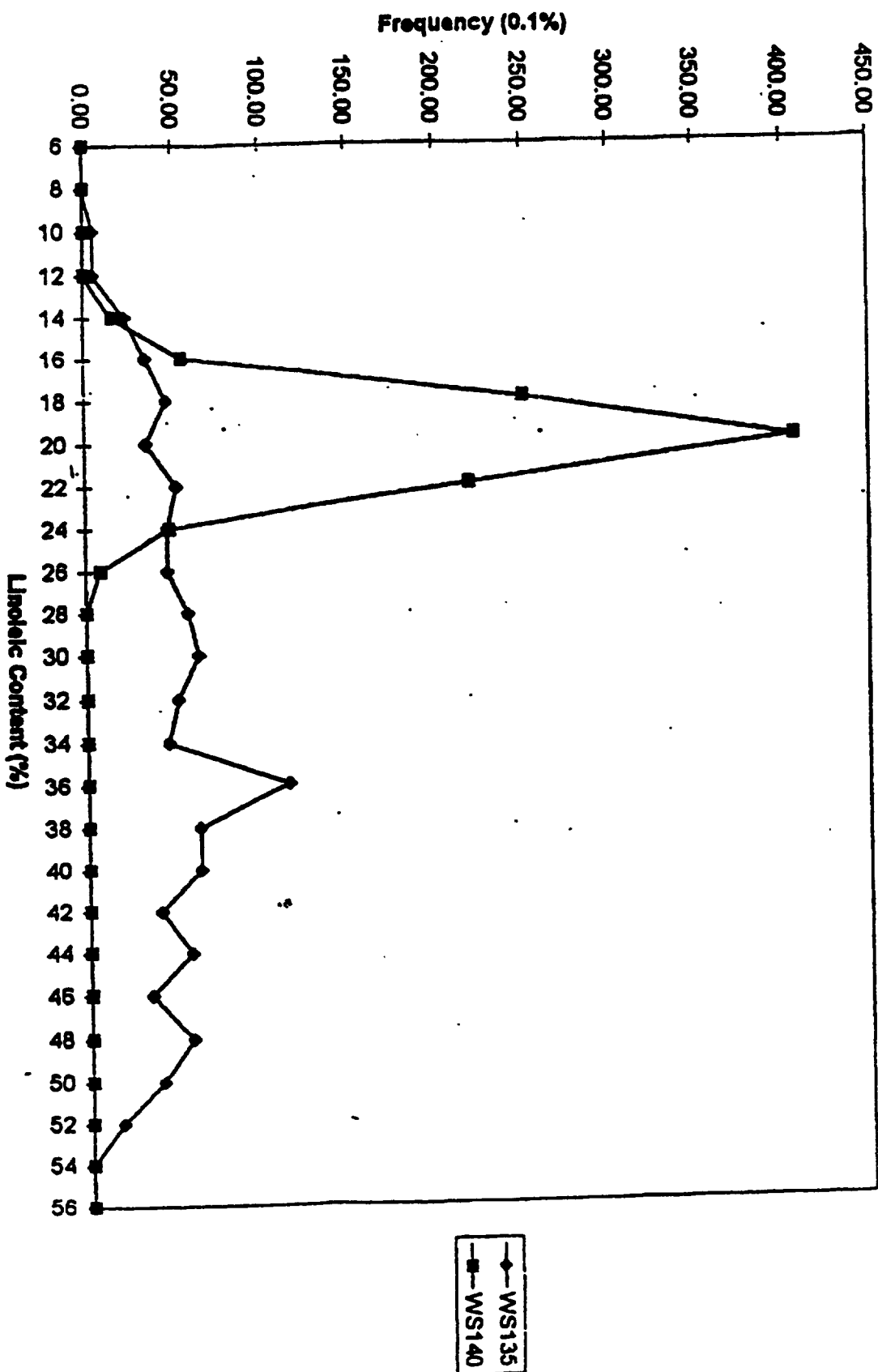


Figure 4

Distribution of T2 Populations with Transformed Fad2 F-Allele Point-Mutation and Fad2 D-Allele Genes, Classified by 18:2 Content



## DECLARATION and POWER OF ATTORNEY

As a below-named inventor, I hereby declare that:

My residence, post office address and citizenship are as stated below next to my name.

I believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled:

**GENES FOR MUTANT MICROSOMAL DELTA-12 FATTY ACID DESATURASES AND  
RELATED ENZYMES FROM PLANTS**

the specification of which is attached hereto unless the following box is checked:

☒ was filed on 10/9/96 as U.S. Application No. 08/728,025 or PCT International Application No. \_\_\_\_\_ and was amended on \_\_\_\_\_ (if applicable).

I hereby state that I have reviewed and understand the contents of the above identified specification, including the claims, as amended by any amendment referred to above.

I acknowledge the duty to disclose information which is known to me to be material to patentability as defined in 37 CFR § 1.56.

I hereby claim foreign priority benefits under 35 U.S.C. § 119(a)-(d) or § 365(b) of any foreign application(s) for patent or inventor's certificate, or § 365(a) of any PCT International application which designated at least one country other than the United States, listed below and have also identified below, by checking the box, any foreign application for patent or inventor's certificate, or PCT International application having a filing date before that of the application on which priority is claimed.

Application No.	Country	Filing Date	Priority Claimed (Yes/No)
-----------------	---------	-------------	---------------------------

I hereby claim the benefit under 35 U.S.C. § 119(e) of any United States Provisional Application(s) listed below.

U.S. Provisional Application No.	U.S. Filing Date
----------------------------------	------------------

I hereby claim the benefit under 35 U.S.C. § 120 of any United States application(s), or § 365(c) of any PCT International Application designating the United States, listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior United States application or PCT International Application in the manner provided by the first paragraph of 35 U.S.C. § 112, I acknowledge the duty to disclose information which is known to me to be material to patentability as defined in 37 CFR § 1.56 which became available between the filing date of the prior application and the national or PCT International filing date of this application.

Application No.	Filing Date	Status (patented, pending or abandoned)
-----------------	-------------	---

**POWER OF ATTORNEY:** I hereby appoint the following attorney(s) and/or agent(s) the power to prosecute this application and transact all business in the Patent and Trademark Office connected therewith:

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I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

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			<b>Zip Code</b> <b>50322</b>

## DECLARATION and POWER OF ATTORNEY

As a below-named inventor, I hereby declare that:

My residence, post office address and citizenship are as stated below next to my name.

I believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled:

**Genes For Mutant Microsomal Delta-12 Fatty Acid Desaturases And Related Enzymes From Plants**

the specification of which is attached hereto unless the following box is checked:

☒ was filed on October 9, 1996 as U.S. Application No. 08/728,025 or PCT International Application No. \_\_\_\_\_  
and was amended on \_\_\_\_\_ (if applicable).

I hereby state that I have reviewed and understand the contents of the above identified specification, including the claims, as amended by any amendment referred to above.

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Application No. \_\_\_\_\_ Country \_\_\_\_\_ Filing Date \_\_\_\_\_ Priority Claimed (Yes/No) \_\_\_\_\_

I hereby claim the benefit under 35 U.S.C. § 119(e) of any United States Provisional Application(s) listed below.

U.S. Provisional Application No. \_\_\_\_\_

U.S. Filing Date \_\_\_\_\_

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Application No. \_\_\_\_\_ Filing Date \_\_\_\_\_ Status (patented, pending or abandoned) \_\_\_\_\_

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I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

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SCAN

**PATENT**  
**EXPRESS LABEL NO.: EL073739282US**

**IN THE UNITED STATES PATENT AND TRADEMARK OFFICE**

IN THE APPLICATION OF:

RICHARD MARTIN BROGLIE ET AL.

CASE NO.: BB1334 USNA CNT1

APPLICATION NO.: UNKNOWN

GROUP ART UNIT: UNKNOWN

FILED: CONCURRENTLY HEREWITH

EXAMINER: UKNNOWN

**FOR: GENES FOR MUTANT MICROSOMAL DELTA-12 FATTY ACID  
DESATURASES AND RELATED ENZYMES FROM PLANTS**

Assistant Commissioner for Patents  
Washington, DC 20231

Sir:

**DECLARATION IN ACCORDANCE WITH 37 CFR 1.821**

I hereby state that the content of the paper and computer readable copies of the Sequence Listing, submitted in accordance with 37 CFR 1.821(c) and (e), respectively are the same.

Respectfully submitted,



LYNNE M. CHRISTENBURY  
ATTORNEY FOR APPLICANTS  
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Dated: 22 August 2000

002280 62524960

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- (ii) TITLE OF INVENTION: GENES FOR MUTANT MICROSOMAL  
FATTY ACID DELTA-12  
DESATURASES AND RELATED  
ENZYMES FROM PLANTS
- (iii) NUMBER OF SEQUENCES: 17
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(E) COUNTRY: U.S.A.  
(F) ZIP: 19898
- (v) COMPUTER READABLE FORM:  
(A) MEDIUM TYPE: FLOPPY DISK  
(B) COMPUTER: IBM PC COMPATIBLE  
(C) OPERATING SYSTEM: MICROSOFT WINDOWS 95  
(D) SOFTWARE: MICROSOFT OFFICE 97
- (vi) CURRENT APPLICATION DATA:  
(A) APPLICATION NUMBER: 09/232,948  
(B) FILING DATE: January 19, 1999  
(C) CLASSIFICATION:
- (vii) PRIOR APPLICATION DATA:  
(A) APPLICATION NUMBER: 08/256,047  
(B) FILING DATE: NOVEMBER 17, 1992
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(2) INFORMATION FOR SEQ ID NO:1:

- (i) SEQUENCE CHARACTERISTICS:  
 (A) LENGTH: 1464 base pairs  
 (B) TYPE: nucleic acid  
 (C) STRANDEDNESS: single  
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

- (ix) FEATURE:  
 (A) NAME/KEY: CDS  
 (B) LOCATION: 130..1281

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

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CCT CAC CCT CTC TCC TAC TTC GCC TGG CCT CTC TAC TGG GCC TGC CAG      408
Pro His Pro Leu Ser Tyr Phe Ala Trp Pro Leu Tyr Trp Ala Cys Gln
              80              85              90

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Gly Cys Val Leu Thr Gly Val Trp Val Ile Ala His Glu Cys Gly His
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CAC GCC TTC AGC GAC TAC CAG TGG CTG GAC GAC ACC GTC GGC CTC ATC      504
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270 275 280 285	
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Ala His His Leu Phe Ser Thr Met Pro His Tyr His Ala Met Glu Ala	
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335 340 345	
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Thr Pro Val Val Lys Ala Met Trp Arg Glu Ala Lys Glu Cys Ile Tyr	
350 355 360 365	
GTG GAA CCG GAC AGG CAA GGT GAG AAG AAA GGT GTG TTC TGG TAC AAC	1272
Val Glu Pro Asp Arg Gln Gly Glu Lys Lys Gly Val Phe Trp Tyr Asn	
370 375 380	
AAT AAG TTA TGAAGCAAAG AAGAACTGA ACCTTTCTCT TCTATGATTG	1321
Asn Lys Leu	
TCTTTGTTTA AGAAGCTATG TTTCTGTTTC AATAATCTTA ATTATCCATT TTGTTGTGTT	1381
TTCTGACATT TTGGCTAAAA TTATGTGATG TTGGAAGTTA GTGTCTAAAA AAAAAAAAAA	1441
AAAAAAAAAA AAAAAAAAAA AAA	1464

[illegible]

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 384 amino acids  
(B) TYPE: amino acid  
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

Met 1	Gly	Ala	Gly	Gly 5	Arg	Met	Gln	Val	Ser 10	Pro	Pro	Ser	Lys	Lys 15	Ser
Glu	Thr	Asp	Asn 20	Ile	Lys	Arg	Val	Pro 25	Cys	Glu	Thr	Pro	Pro 30	Phe	Thr
Val	Gly	Glu 35	Leu	Lys	Lys	Ala	Ile 40	Pro	Pro	His	Cys	Phe 45	Lys	Arg	Ser
Ile	Pro 50	Arg	Ser	Phe	Ser	Tyr 55	Leu	Ile	Trp	Asp	Ile 60	Ile	Ile	Ala	Ser
Cys 65	Phe	Tyr	Tyr	Val	Ala 70	Thr	Thr	Tyr	Phe	Pro 75	Leu	Leu	Pro	His	Pro 80
Leu	Ser	Tyr	Phe	Ala 85	Trp	Pro	Leu	Tyr	Trp 90	Ala	Cys	Gln	Gly	Cys 95	Val
Leu	Thr	Gly	Val 100	Trp	Val	Ile	Ala	His 105	Glu	Cys	Gly	His	His 110	Ala	Phe
Ser	Asp	Tyr 115	Gln	Trp	Leu	Asp	Asp 120	Thr	Val	Gly	Leu	Ile 125	Phe	His	Ser
Phe	Leu 130	Leu	Val	Pro	Tyr	Phe 135	Ser	Trp	Lys	Tyr	Ser 140	His	Arg	Arg	His
His 145	Ser	Asn	Thr	Gly	Ser 150	Leu	Glu	Arg	Asp	Glu 155	Val	Phe	Val	Pro	Lys 160
Lys	Lys	Ser	Asp	Ile 165	Lys	Trp	Tyr	Gly	Lys 170	Tyr	Leu	Asn	Asn	Pro 175	Leu
Gly	Arg	Thr	Val 180	Met	Leu	Thr	Val	Gln 185	Phe	Thr	Leu	Gly	Trp 190	Pro	Leu
Tyr	Leu	Ala 195	Phe	Asn	Val	Ser	Gly 200	Arg	Pro	Tyr	Asp	Gly 205	Gly	Phe	Ala
Cys	His 210	Phe	His	Pro	Asn	Ala 215	Pro	Ile	Tyr	Asn	Asp 220	Arg	Glu	Arg	Leu
Gln 225	Ile	Tyr	Ile	Ser	Asp 230	Ala	Gly	Ile	Leu	Ala 235	Val	Cys	Tyr	Gly	Leu 240
Tyr	Arg	Tyr	Ala	Ala 245	Val	Gln	Gly	Val	Ala 250	Ser	Met	Val	Cys	Phe 255	Tyr
Gly	Val	Pro	Leu 260	Leu	Ile	Val	Asn	Gly 265	Phe	Leu	Val	Leu	Ile 270	Thr	Tyr
Leu	Gln	His	Thr	His	Pro	Ser	Leu	Pro	His	Tyr	Asp	Ser	Ser	Glu	Trp



TGC Cys 65	TTC Phe	TAC Tyr	TAC Tyr	GTC Val	GCC Ala 70	ACC Thr	ACT Thr	TAC Tyr	TTC Phe	CCT Pro 75	CTC Leu	CTC Leu	CCT Pro	CAC His	CCT Pro 80	240
CTC Leu	TCC Ser	TAC Tyr	TTC Phe	GCC Ala 85	TGG Trp	CCT Pro	CTC Leu	TAC Tyr	TGG Trp 90	GCC Ala	TGC Cys	CAG Gln	GGC Gly	TGC Cys 95	GTC Val	288
CTA Leu	ACC Thr	GGC Gly	GTC Val 100	TGG Trp	GTC Val	ATA Ile	GCC Ala	CAC His 105	AAG Lys	TGC Cys	GGC Gly	CAC His	CAC His 110	GCC Ala	TTC Phe	336
AGC Ser	GAC Asp	TAC Tyr 115	CAG Gln	TGG Trp	CTG Leu	GAC Asp	GAC Asp 120	ACC Thr	GTC Val	GGC Gly	CTC Leu	ATC Ile 125	TTC Phe	CAC His	TCC Ser	384
TTC Phe 130	CTC Leu	CTC Leu	GTC Val	CCT Pro	TAC Tyr	TTC Phe 135	TCC Ser	TGG Trp	AAG Lys	TAC Tyr	AGT Ser 140	CAT His	CGA Arg	CGC Arg	CAC His	432
CAT His 145	TCC Ser	AAC Asn	ACT Thr	GGC Gly	TCC Ser 150	CTC Leu	GAG Glu	AGA Arg	GAC Asp	GAA Glu 155	GTG Val	TTT Phe	GTC Val	CCC Pro	AAG Lys 160	480
AAG Lys	AAG Lys	TCA Ser	GAC Asp	ATC Ile 165	AAG Lys	TGG Trp	TAC Tyr	GGC Gly	AAG Lys 170	TAC Tyr	CTC Leu	AAC Asn	AAC Asn	CCT Pro 175	TTG Leu	528
GGA Gly	CGC Arg	ACC Thr	GTG Val 180	ATG Met	TTA Leu	ACG Thr	GTT Val	CAG Gln 185	TTC Phe	ACT Thr	CTC Leu	GGC Gly	TGG Trp 190	CCT Pro	TTG Leu	576
TAC Tyr	TTA Leu	GCC Ala 195	TTC Phe	AAC Asn	GTC Val	TCG Ser	GGG Gly 200	AGA Arg	CCT Pro	TAC Tyr	GAC Asp	GGC Gly 205	GGC Gly	TTC Phe	GCT Ala	624
TGC Cys 210	CAT His	TTC Phe	CAC His	CCC Pro	AAC Asn	GCT Ala 215	CCC Pro	ATC Ile	TAC Tyr	AAC Asn	GAC Asp 220	CGC Arg	GAG Glu	CGT Arg	CTC Leu	672
CAG Gln 225	ATA Ile	TAC Tyr	ATC Ile	TCC Ser	GAC Asp 230	GCT Ala	GGC Gly	ATC Ile	CTC Leu	GCC Ala 235	GTG Val	TGC Cys	TAC Tyr	GGT Gly	CTC Leu 240	720
TAC Tyr	CGC Arg	TAC Tyr	GCT Ala	GCT Ala 245	GTC Val	CAA Gln	GGA Gly	GTT Val	GCC Ala 250	TCG Ser	ATG Met	GTC Val	TGC Cys	TTC Phe 255	TAC Tyr	768
GGA Gly	GTT Val	CCG Pro	CTT Leu 260	CTG Leu	ATT Ile	GTC Val	AAT Asn	GGG Gly 265	TTC Phe	TTA Leu	GTT Val	TTG Leu	ATC Ile 270	ACT Thr	TAC Tyr	816
TTG Leu	CAG Gln	CAC His 275	ACG Thr	CAT His	CCT Pro	TCC Ser	CTG Leu 280	CCT Pro	CAC His	TAT Tyr	GAC Asp	TCG Ser 285	TCT Ser	GAG Glu	TGG Trp	864
GAT Asp	TGG Trp 290	TTG Leu	AGG Arg	GGA Gly	GCT Ala	TTG Leu 295	GCC Ala	ACC Thr	GTT Val	GAC Asp	AGA Arg 300	GAC Asp	TAC Tyr	GGA Gly	ATC Ile	912
TTG Leu 305	AAC Asn	AAG Lys	GTC Val	TTC Phe 310	CAC His	AAT Asn	ATC Ile	ACG Thr	GAC Asp	ACG Thr 315	CAC His	GTG Val	GCG Ala	CAT His	CAC His 320	960



Gly Arg Thr Val Met Leu Thr Val Gln Phe Thr Leu Gly Trp Pro Leu  
 180 185 190  
 Tyr Leu Ala Phe Asn Val Ser Gly Arg Pro Tyr Asp Gly Gly Phe Ala  
 195 200 205  
 Cys His Phe His Pro Asn Ala Pro Ile Tyr Asn Asp Arg Glu Arg Leu  
 210 215 220  
 Gln Ile Tyr Ile Ser Asp Ala Gly Ile Leu Ala Val Cys Tyr Gly Leu  
 225 230 235 240  
 Tyr Arg Tyr Ala Ala Val Gln Gly Val Ala Ser Met Val Cys Phe Tyr  
 245 250 255  
 Gly Val Pro Leu Leu Ile Val Asn Gly Phe Leu Val Leu Ile Thr Tyr  
 260 265 270  
 Leu Gln His Thr His Pro Ser Leu Pro His Tyr Asp Ser Ser Glu Trp  
 275 280 285  
 Asp Trp Leu Arg Gly Ala Leu Ala Thr Val Asp Arg Asp Tyr Gly Ile  
 290 295 300  
 Leu Asn Lys Val Phe His Asn Ile Thr Asp Thr His Val Ala His His  
 305 310 315 320  
 Leu Phe Ser Thr Met Pro His Tyr His Ala Met Glu Ala Thr Lys Ala  
 325 330 335  
 Ile Lys Pro Ile Leu Gly Glu Tyr Tyr Gln Phe Asp Gly Thr Pro Val  
 340 345 350  
 Val Lys Ala Met Trp Arg Glu Ala Lys Glu Cys Ile Tyr Val Glu Pro  
 355 360 365  
 Asp Arg Gln Gly Glu Lys Lys Gly Val Phe Trp Tyr Asn Asn Lys Leu  
 370 375 380

(2) INFORMATION FOR SEQ ID NO:5:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 1155 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:

- (A) ORGANISM: Brassica napus

(ix) FEATURE:

- (D) OTHER INFORMATION: Wild type F form.

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

ATG GGT GCA GGT GGA AGA ATG CAA GTG TCT CCT CCC TCC AAG AAG TCT  
 Met Gly Ala Gly Gly Arg Met Gln Val Ser Pro Pro Ser Lys Lys Ser

48

1			5			10			15							
GAA Glu	ACC Thr	GAC Asp	ACC Thr 20	ATC Ile	AAG Lys	CGC Arg	GTA Val	CCC Pro 25	TGC Cys	GAG Glu	ACA Thr	CCG Pro	CCC Pro 30	TTC Phe	ACT Thr	96
GTC Val	GGA Gly 35	GAA Glu	CTC Leu	AAG Lys	AAA Lys	GCA Ala	ATC Ile 40	CCA Pro	CCG Pro	CAC His	TGT Cys	TTC Phe 45	AAA Lys	CGC Arg	TCG Ser	144
ATC Ile	CCT Pro 50	CGC Arg	TCT Ser	TTC Phe	TCC Ser	TAC Tyr 55	CTC Leu	ATC Ile	TGG Trp	GAC Asp 60	ATC Ile	ATC Ile	ATA Ile	GCC Ala	TCC Ser	192
TGC Cys 65	TTC Phe	TAC Tyr	TAC Tyr	GTC Val	GCC Ala 70	ACC Thr	ACT Thr	TAC Tyr	TTC Phe	CCT Pro 75	CTC Leu	CTC Leu	CCT Pro	CAC His	CCT Pro 80	240
CTC Leu	TCC Ser	TAC Tyr	TTC Phe	GCC Ala 85	TGG Trp	CCT Pro	CTC Leu	TAC Tyr	TGG Trp 90	GCC Ala	TGC Cys	CAA Gln	GGG Gly	TGC Cys 95	GTC Val	288
CTA Leu	ACC Thr	GGC Gly	GTC Val 100	TGG Trp	GTC Val	ATA Ile	GCC Ala	CAC His 105	GAG Glu	TGC Cys	GGC Gly	CAC His	CAC His 110	GCC Ala	TTC Phe	336
AGC Ser	GAC Asp	TAC Tyr 115	CAG Gln	TGG Trp	CTT Leu	GAC Asp 120	GAC Asp	ACC Thr	GTC Val	GGT Gly	CTC Leu	ATC Ile 125	TTC Phe	CAC His	TCC Ser	384
TTC Phe 130	CTC Leu	CTC Leu	GTC Val	CCT Pro	TAC Tyr	TTC Phe 135	TCC Ser	TGG Trp	AAG Lys	TAC Tyr 140	AGT Ser	CAT His	CGC Arg	AGC Ser	CAC His	432
CAT His 145	TCC Ser	AAC Asn	ACT Thr	GGC Gly	TCC Ser 150	CTC Leu	GAG Glu	AGA Arg	GAC Asp	GAA Glu 155	GTG Val	TTT Phe	GTC Val	CCC Pro	AAG Lys 160	480
AAG Lys	AAG Lys	TCA Ser	GAC Asp 165	ATC Ile	AAG Lys	TGG Trp	TAC Tyr	GGC Gly 170	AAG Lys	TAC Tyr	CTC Leu	AAC Asn	AAC Asn	CCT Pro 175	TTG Leu	528
GGA Gly	CGC Arg	ACC Thr 180	GTG Val	ATG Met	TTA Leu	ACG Thr	GTT Val	CAG Gln 185	TTC Phe	ACT Thr	CTC Leu	GGC Gly 190	TGG Trp	CCG Pro	TTG Leu	576
TAC Tyr	TTA Leu 195	GCC Ala	TTC Phe	AAC Asn	GTC Val	TCG Ser	GGA Gly 200	AGA Arg	CCT Pro	TAC Tyr	GAC Asp 205	GGC Gly 205	GGC Gly	TTC Phe	CGT Arg	624
TGC Cys 210	CAT His	TTC Phe	CAC His	CCC Pro	AAC Asn	GCT Ala 215	CCC Pro	ATC Ile	TAC Tyr	AAC Asn 220	GAC Asp	CGC Arg	GAG Glu	CGT Arg	CTC Leu	672
CAG Gln 225	ATA Ile	TAC Tyr	ATC Ile	TCC Ser	GAC Asp 230	GCT Ala	GGC Gly	ATC Ile	CTC Leu	GCC Ala 235	GTG Val	TGC Cys	TAC Tyr	GGT Gly	CTC Leu 240	720
TTC Phe	CGT Arg	TAC Tyr	GCC Ala	GCC Ala 245	GGC Gly	CAG Gln	GGA Gly	GTG Val	GCC Ala 250	TCG Ser	ATG Met	GTC Val	TGC Cys	TTC Phe 255	TAC Tyr	768
GGG Gly	GTC Gly	CCG Gly	CTT Gly	CTG Gly	ATT Gly	GTC Gly	AAT Gly	GGT Gly	TTC Gly	CTC Gly	GTG Gly	TTG Gly	ATC Gly	ACT Gly	TAC Gly	816

Gly	Val	Pro	Leu	Leu	Ile	Val	Asn	Gly	Phe	Leu	Val	Leu	Ile	Thr	Tyr		
			260					265					270				
TTG	CAG	CAC	ACG	CAT	CCT	TCC	CTG	CCT	CAC	TAC	GAT	TCG	TCC	GAG	TGG	864	
Leu	Gln	His	Thr	His	Pro	Ser	Leu	Pro	His	Tyr	Asp	Ser	Ser	Glu	Trp		
		275					280					285					
GAT	TGG	TTC	AGG	GGA	GCT	TTG	GCT	ACC	GTT	GAC	AGA	GAC	TAC	GGA	ATC	912	
Asp	Trp	Phe	Arg	Gly	Ala	Leu	Ala	Thr	Val	Asp	Arg	Asp	Tyr	Gly	Ile		
	290					295					300						
TTG	AAC	AAG	GTC	TTC	CAC	AAT	ATT	ACC	GAC	ACG	CAC	GTG	GCC	CAT	CAT	960	
Leu	Asn	Lys	Val	Phe	His	Asn	Ile	Thr	Asp	Thr	His	Val	Ala	His	His		
305					310					315				320			
CCG	TTC	TCC	ACG	ATG	CCG	CAT	TAT	CAC	GCG	ATG	GAA	GCT	ACC	AAG	GCG	1008	
Pro	Phe	Ser	Thr	Met	Pro	His	Tyr	His	Ala	Met	Glu	Ala	Thr	Lys	Ala		
				325					330					335			
ATA	AAG	CCG	ATA	CTG	GGA	GAG	TAT	TAT	CAG	TTC	GAT	GGG	ACG	CCG	GTG	1056	
Ile	Lys	Pro	Ile	Leu	Gly	Glu	Tyr	Tyr	Gln	Phe	Asp	Gly	Thr	Pro	Val		
			340					345					350				
GTT	AAG	GCG	ATG	TGG	AGG	GAG	GCG	AAG	GAG	TGT	ATC	TAT	GTG	GAA	CCG	1104	
Val	Lys	Ala	Met	Trp	Arg	Glu	Ala	Lys	Glu	Cys	Ile	Tyr	Val	Glu	Pro		
		355					360					365					
GAC	AGG	CAA	GGT	GAG	AAG	AAA	GGT	GTG	TTC	TGG	TAC	AAC	AAT	AAG	TTA	T	1153
Asp	Arg	Gln	Gly	Glu	Lys	Lys	Gly	Val	Phe	Trp	Tyr	Asn	Asn	Lys	Leu		
	370					375					380						
GA																	1155

(2) INFORMATION FOR SEQ ID NO:6:

- (i) SEQUENCE CHARACTERISTICS:  
 (A) LENGTH: 384 amino acids  
 (B) TYPE: amino acid  
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

Met	Gly	Ala	Gly	Gly	Arg	Met	Gln	Val	Ser	Pro	Pro	Ser	Lys	Lys	Ser
1				5					10					15	
Glu	Thr	Asp	Thr	Ile	Lys	Arg	Val	Pro	Cys	Glu	Thr	Pro	Pro	Phe	Thr
		20					25					30			
Val	Gly	Glu	Leu	Lys	Lys	Ala	Ile	Pro	Pro	His	Cys	Phe	Lys	Arg	Ser
		35				40						45			
Ile	Pro	Arg	Ser	Phe	Ser	Tyr	Leu	Ile	Trp	Asp	Ile	Ile	Ile	Ala	Ser
	50					55				60					
Cys	Phe	Tyr	Tyr	Val	Ala	Thr	Thr	Tyr	Phe	Pro	Leu	Leu	Pro	His	Pro
	65				70				75					80	
Leu	Ser	Tyr	Phe	Ala	Trp	Pro	Leu	Tyr	Trp	Ala	Cys	Gln	Gly	Cys	Val
			85					90					95		
Leu	Thr	Gly	Val	Trp	Val	Ile	Ala	His	Glu	Cys	Gly	His	His	Ala	Phe





(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:  
(A) ORGANISM: Brassica napus

(vii) IMMEDIATE SOURCE:  
(B) CLONE: IMC Q508

(ix) FEATURE:  
(D) OTHER INFORMATION: T to A transversion  
mutation at nucleotide 515  
of the F form

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

ATG GGT GCA GGT GGA AGA ATG CAA GTG TCT CCT CCC TCC AAG AAG TCT	48
Met Gly Ala Gly Gly Arg Met Gln Val Ser Pro Pro Ser Lys Lys Ser	
1 5 10 15	
GAA ACC GAC ACC ATC AAG CGC GTA CCC TGC GAG ACA CCG CCC TTC ACT	96
Glu Thr Asp Thr Ile Lys Arg Val Pro Cys Glu Thr Pro Pro Phe Thr	
20 25 30	
GTC GGA GAA CTC AAG AAA GCA ATC CCA CCG CAC TGT TTC AAA CGC TCG	144
Val Gly Glu Leu Lys Lys Ala Ile Pro Pro His Cys Phe Lys Arg Ser	
35 40 45	
ATC CCT CGC TCT TTC TCC TAC CTC ATC TGG GAC ATC ATC ATA GCC TCC	192
Ile Pro Arg Ser Phe Ser Tyr Leu Ile Trp Asp Ile Ile Ile Ala Ser	
50 55 60	
TGC TTC TAC TAC GTC GCC ACC ACT TAC TTC CCT CTC CTC CCT CAC CCT	240
Cys Phe Tyr Tyr Val Ala Thr Thr Tyr Phe Pro Leu Leu Pro His Pro	
65 70 75 80	
CTC TCC TAC TTC GCC TGG CCT CTC TAC TGG GCC TGC CAA GGG TGC GTC	288
Leu Ser Tyr Phe Ala Trp Pro Leu Tyr Trp Ala Cys Gln Gly Cys Val	
85 90 95	
CTA ACC GGC GTC TGG GTC ATA GCC CAC GAG TGC GGC CAC CAC GCC TTC	336
Leu Thr Gly Val Trp Val Ile Ala His Glu Cys Gly His His Ala Phe	
100 105 110	
AGC GAC TAC CAG TGG CTT GAC GAC ACC GTC GGT CTC ATC TTC CAC TCC	384
Ser Asp Tyr Gln Trp Leu Asp Asp Thr Val Gly Leu Ile Phe His Ser	
115 120 125	
TTC CTC CTC GTC CCT TAC TTC TCC TGG AAG TAC AGT CAT CGC AGC CAC	432
Phe Leu Leu Val Pro Tyr Phe Ser Trp Lys Tyr Ser His Arg Ser His	
130 135 140	
CAT TCC AAC ACT GGC TCC CTC GAG AGA GAC GAA GTG TTT GTC CCC AAG	480
His Ser Asn Thr Gly Ser Leu Glu Arg Asp Glu Val Phe Val Pro Lys	
145 150 155 160	
AAG AAG TCA GAC ATC AAG TGG TAC GGC AAG TAC CAC AAC AAC CCT TTG	528
Lys Lys Ser Asp Ile Lys Trp Tyr Gly Lys Tyr His Asn Asn Pro Leu	
165 170 175	
GGA CGC ACC GTG ATG TTA ACG GTT CAG TTC ACT CTC GGC TGG CCG TTG	576
Gly Arg Thr Val Met Leu Thr Val Gln Phe Thr Leu Gly Trp Pro Leu	

190

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

Met 1	Gly	Ala	Gly	Gly 5	Arg	Met	Gln	Val	Ser 10	Pro	Pro	Ser	Lys	Lys 15	Ser
Glu	Thr	Asp	Thr 20	Ile	Lys	Arg	Val	Pro 25	Cys	Glu	Thr	Pro	Pro 30	Phe	Thr
Val	Gly	Glu 35	Leu	Lys	Lys	Ala	Ile 40	Pro	Pro	His	Cys	Phe 45	Lys	Arg	Ser
Ile	Pro 50	Arg	Ser	Phe	Ser	Tyr 55	Leu	Ile	Trp	Asp	Ile 60	Ile	Ile	Ala	Ser
Cys 65	Phe	Tyr	Tyr	Val	Ala 70	Thr	Thr	Tyr	Phe	Pro 75	Leu	Leu	Pro	His	Pro 80
Leu	Ser	Tyr	Phe	Ala 85	Trp	Pro	Leu	Tyr	Trp 90	Ala	Cys	Gln	Gly	Cys 95	Val
Leu	Thr	Gly	Val 100	Trp	Val	Ile	Ala	His 105	Glu	Cys	Gly	His	His 110	Ala	Phe
Ser	Asp	Tyr 115	Gln	Trp	Leu	Asp	Asp 120	Thr	Val	Gly	Leu	Ile 125	Phe	His	Ser
Phe	Leu 130	Leu	Val	Pro	Tyr	Phe 135	Ser	Trp	Lys	Tyr	Ser 140	His	Arg	Ser	His
His 145	Ser	Asn	Thr	Gly	Ser 150	Leu	Glu	Arg	Asp	Glu 155	Val	Phe	Val	Pro	Lys 160
Lys	Lys	Ser	Asp	Ile 165	Lys	Trp	Tyr	Gly	Lys 170	Tyr	His	Asn	Asn	Pro 175	Leu
Gly	Arg	Thr	Val 180	Met	Leu	Thr	Val	Gln 185	Phe	Thr	Leu	Gly	Trp 190	Pro	Leu
Tyr	Leu	Ala 195	Phe	Asn	Val	Ser	Gly 200	Arg	Pro	Tyr	Asp	Gly 205	Gly	Phe	Arg
Cys	His 210	Phe	His	Pro	Asn	Ala 215	Pro	Ile	Tyr	Asn	Asp 220	Arg	Glu	Arg	Leu
Gln 225	Ile	Tyr	Ile	Ser	Asp 230	Ala	Gly	Ile	Leu	Ala 235	Val	Cys	Tyr	Gly	Leu 240
Phe	Arg	Tyr	Ala	Ala 245	Gly	Gln	Gly	Val	Ala 250	Ser	Met	Val	Cys	Phe 255	Tyr
Gly	Val	Pro	Leu 260	Leu	Ile	Val	Asn	Gly 265	Phe	Leu	Val	Leu	Ile 270	Thr	Tyr
Leu	Gln	His 275	Thr	His	Pro	Ser	Leu 280	Pro	His	Tyr	Asp	Ser 285	Ser	Glu	Trp
Asp	Trp 290	Phe	Arg	Gly	Ala	Leu 295	Ala	Thr	Val	Asp	Arg 300	Asp	Tyr	Gly	Ile
Leu 305	Asn	Lys	Val	Phe	His 310	Asn	Ile	Thr	Asp	Thr 315	His	Val	Ala	His	His 320
Pro	Phe	Ser	Thr	Met 325	Pro	His	Tyr	His	Ala 330	Met	Glu	Ala	Thr	Lys 335	Ala

Ile Lys Pro Ile Leu Gly Glu Tyr Tyr Gln Phe Asp Gly Thr Pro Val  
340 345 350  
Val Lys Ala Met Trp Arg Glu Ala Lys Glu Cys Ile Tyr Val Glu Pro  
355 360 365  
Asp Arg Gln Gly Glu Lys Lys Gly Val Phe Trp Tyr Asn Asn Lys Leu  
370 375 380

(2) INFORMATION FOR SEQ ID NO:9:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 23 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

CATGGGTGCA GGTGAAGAA TGC

23

(2) INFORMATION FOR SEQ ID NO:10:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 21 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

GTTTCTTCTT TGCTTCATAA C

21

(2) INFORMATION FOR SEQ ID NO:11:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 23 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:

CATGGGTGCA GGTGAAGAA TGC

23

(2) INFORMATION FOR SEQ ID NO:12:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 21 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:12:

TCTTTCACCA TCATCATATC C

21

(2) INFORMATION FOR SEQ ID NO:13:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 19 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:13:

GTCTGGGTCA TAGCCCACG

19

(2) INFORMATION FOR SEQ ID NO:14:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 19 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:14:

GTCTGGGTCA TAGCCCACA

19

(2) INFORMATION FOR SEQ ID NO:15:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 17 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:15:

CTGGGTCATA GCCCATG

17

(2) INFORMATION FOR SEQ ID NO:16:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 17 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:16:

CTGGGTCATA GCCCACA

17

(2) INFORMATION FOR SEQ ID NO:17:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 5 amino acids
  - (B) TYPE: amino acid

(C) STRANDEDNESS:  
 (D) TOPOLOGY: linear  
  
 (ii) MOLECULE TYPE: peptide  
 (iii) HYPOTHETICAL: YES  
 (iv) ANTI-SENSE: NO  
 (v) FRAGMENT TYPE: internal  
 (ix) FEATURE:  
     (A) NAME/KEY: Modified-site  
     (B) LOCATION: 2  
     (D) OTHER INFORMATION: /product= "Asp or Glu"  
  
 (ix) FEATURE:  
     (A) NAME/KEY: Modified-site  
     (B) LOCATION: 4  
     (D) OTHER INFORMATION: /product= "Ala or Gly"  
  
 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:17:  
  
 His Xaa Cys Xaa His  
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